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Neoplasm Studies

IX. The Effects in Tissue Culture of N,N-Dimethyl-*p*-Phenylenediamine on Rat Liver Tumors Induced by *p*-Dimethylaminoazobenzene*

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Stevenson, Dobriner, and Rhoads (12) have reported that *p*-dimethylaminoazobenzene is metabolized by the rat to produce several aniline and *p*-phenylenediamine derivatives. These split products have been tested on certain enzyme systems by Kensler, Dexter, and Rhoads (5), by Kensler (13), and by Potter (11). One of the probable intermediary products, N,N-dimethyl-*p*-phenylenediamine, produces a strong inhibition of the activity of the diphosphopyridine nucleotide (coenzyme I) system (5) and of other sulfhydryl enzymes (11, 13). Other split products, with the exception of *p*-phenylenediamine, are either ineffective or produce relatively slight inhibitions of the activity of the diphosphopyridine nucleotide system.

Kensler, Young, and Rhoads (personal communication) found that the autoxidations occurring in a normal rat liver cell-free brei (ground tissue suspension) were also inhibited by N,N-dimethyl-*p*-phenylenediamine, whereas those occurring in a liver tumor brei were not inhibited significantly. However, when tissue slices were used the inhibition of the respiration of liver tissues was not appreciably greater than that of the liver tumor.

The experiments described in this report were undertaken to determine the effect of N,N-dimethyl-*p*-phenylenediamine on normal and on tumor liver epithelium in tissue cultures. Such procedures permit, by direct observation, the evaluation of toxic action on cells that are actively growing and identifiable.

PROCEDURES

Preparation of Tissues

The livers of rats,[1] either normal or those fed on diets containing *p*-dimethylaminoazobenzene, were removed under aseptic conditions and fragments dissected for culturing. Small pieces, of about 1 cu. mm., were cut in Tyrode solution and each fragment was thoroughly washed. This is essential for normal liver, since the fragmentation of the tissue liberates granules, salts, and liver secretion products that inhibit growth. The tissues were embedded in a drop of medium on a small circular cover slip attached to a larger square (mica) cover slip by a film of fluid according to the double cover slip method (2).

The normal medium consisted of 2 drops of a mixture of fowl plasma, 1 part by volume; rat serum, 3 parts; and chick embryo extract, 1 part. For the reactivity tests, the medium was modified by replacing one of the 3 parts of serum with Tyrode solution containing N,N-dimethyl-*p*-phenylenediamine.

Normal adult liver epithelium grew slowly and only after a latent period of at least 24 to 48 hours. During this period numerous wandering cells (Kupffer cells and a few fibroblasts) migrated into the medium. About 50 per cent of the normal liver cultures produced good epithelial growth in from 72 to 96 hours and these were selected for the experiments (4).

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[1] The rats were prepared for us under the direction of Mr. C. J. Kensler and Dr. C. P. Rhoads, at the Memorial Hospital.

The epithelial cells that grow from liver fragments in tissue culture are of two kinds. One type—from the glandular portion—forms blunt, sheet-like outgrowths composed of large, heavily granulated cells (Fig. 1). Many contain globules (presumably fat) and, in older cultures, yellow pigment granules. The hyaline nuclei are particularly prominent in the flattened cells owing to the contrast between them and the highly granular cytoplasm. The second type, from the duct epithelium, forms finger-like outgrowths of clear, nongranular cells (Figs. 4 and 5), which in older cultures frequently form cysts.

Cultures of liver tumor grew more quickly than normal liver and exhibited a latent period of only a few hours. They were usually ready for the tests after about 48 hours of culturing. By this time most of the Kupffer cells had emigrated well beyond the growing sheets of epithelium. The culture medium, in common with that of all active neoplasms, undergoes considerable liquefaction. Many of the cells within the sheets organize to form duct-like growths and cysts (Figs. 4 and 5). The latter may appear in cultures of 3 to 5 days, and increase in size and number with the age of the culture.

The majority of the tumors were found to be of the cholangioma (duct cell) type and, from cultured fragments, a luxuriant growth of hyaline epithelium was obtained. The epithelial cells of these tumors appear to be structurally similar to those that form ducts and cysts in normal liver cultures. The similarity between the normal duct epithelium and the typical, *p*-dimethylaminoazobenzene-induced tumor epithelium is even more striking when the effects of the N,N-dimethyl-*p*-phenylenediamine compound on the two tissues are analyzed. A few mixed tumors were used that on culturing resulted in a growth of both glandular and ductal epithelium. One or two were obtained from which tissue cultures developed growths of the large, granular parenchymatous cells only.

Preparation of N,N-Dimethyl-*p*-Phenylenediamine-Tyrode Solutions

N,N-Dimethyl-*p*-phenylenediamine is unstable in aqueous solution. It was therefore added to the Tyrode solution only a few minutes before use. If the compound is to be effective its oxidation must not exceed the incipient pink color that appears within 5 minutes after solution in Tyrode. Since the Tyrode component of the culture medium is one-fifth of the whole, the dimethyl compound in the Tyrode solution was prepared at a concentration 5 times that desired for the experiment. The concentration stated for a given series of experiments is that actually present after dilution of the N,N-dimethyl-*p*-phenylenediamine-Tyrode solution by the other components of the culture medium.

Preparation of the Tissues for Comparative Studies

The difference in growth rates of the normal and tumor liver cultures was such that it required about twice as long for normal liver epithelium to attain the dimensions of the epithelial outgrowth of tumor liver. Therefore, in order to have analogous outgrowths for the comparative studies of tumor and normal liver, the dimethyl compound was applied to normal liver cultures from 72 to 96 hours after the cultures were prepared, while the tumor cultures were ready within 48 hours. In some cases, however, the tumor preparations had been subcultured for over 2 months before the dimethyl compound was applied. It has been our experience with tissue cultures in general that the reactivity of the outgrowths is similar irrespective of whether they are obtained from explants of early or late subculturing. The experiments were regularly performed by exposing normal and tumor growths simultaneously to the experimental media.

General Effects of Toxic Agents on Tissue Cultures

These may be classified as follows:—

Inhibitory (reversible).—Growth slows down and stops but resumes on return to normal conditions.

Tissue disorganization.—Epithelial sheets become disorganized and their constituent cells round up and separate from one another, a process resulting in complete breakdown of the sheet structure. This may be reversible providing the toxic agent is removed in time.

Inhibitory (irreversible).—Growth stops permanently. This stage leads to deterioration of the cells.

The following morphological changes (indicating irreversible effects and deterioration) apply more specifically to cultures of liver tissues:—

Cellular deterioration.—Structural changes occurring within the cells: (a) granulation of cytoplasm, (b) appearance of vacuoles in cytoplasm, (c) accumulation of fat globules in cytoplasm, and (d) concurrent or independent changes in nuclei such as an increase in granulation. Some of these changes may be reversible but usually they are not. Differences in susceptibility occur, with the disintegration of some types of cells and the continued growth of others.

The recognizable irreversible changes are of two kinds: (a) fixation, a type of coagulation in which the cells retain their general form but are, nevertheless, dead; and (b) disintegration of the cells.

RESULTS

In the exploratory work, concentrations ranging from 0.0001 to 0.01 *M* were used. Concentrations of 0.004 *M* and higher produced striking changes in both normal and tumor tissues. On the other hand, con-

centrations ranging from 0.0001 to 0.0005 *M* produced definite changes in normal tissues and varying results among different specimens of the tumors.

At first, there appeared to be some variability in effects produced on normal epithelium. It was then found that the presence of two kinds of epithelium, *i. e.* ductal and glandular, was the cause of the apparent variability. A constant susceptibility was obtained on glandular epithelium, while the ductal epithelium, in spite of the formation of intracellular fat drops (Fig. 6), continued to grow and thus showed a resistant reaction to N,N-dimethyl-*p*-phenylenediamine when administered in effective concentrations. The apparent variability observed in normal epithelium is, therefore, significant and understandable.

The survival of the two kinds of normal liver epithelium in the N,N-dimethyl-*p*-phenylenediamine-Tyrode solutions was the following:

Concentration of N,N-dimethyl-*p*-phenylenediamine	Glandular epithelium	Ductal epithelium
0.0001 *M*	Some cells killed	All cells survived
0.0005 *M*	More cells killed	" " "
0.001 *M*	All cells killed	" " "
0.002 *M*	" " "	Some cells killed
0.004 *M*	" " "	More cells killed
0.005 *M*	" " "	All cells killed

The critical concentration of the dimethyl compound for glandular epithelium is therefore 0.0001 *M*, while that for ductal epithelium ranges from 0.002 to 0.004 *M*. The clearest differential was found at a concentration of 0.001 *M*, in which all glandular epithelium is destroyed while the ductal epithelium survives and grows. The results obtained were based on an analysis of over 400 preparations (about 800 fragments) of normal liver tissue.

The tumor studies were based on 520 cultures (about 1,000 individual fragments). The majority of the tumors from rats fed *p*-dimethylaminoazobenzene were found by us to be of the cholangioma, duct cell, type. These cultures proved remarkably resistant to the dimethyl compound. A concentration of 0.001 *M*, which was toxic and produced irreversible changes in normal cultures of glandular epithelium, exerted little or no effect on the outgrowths of the cholangioma cultures.

Occasionally, liver tumors were found that consisted of small, soft nodules of tissue resembling normal liver. Fragments of these nodules in tissue culture showed a predominance of glandular epithelium and Kupffer cells. The outgrowing sheets of the glandular epithelium from these fragments were, with few exceptions, killed after 24 hours' exposure to 0.001 *M* N,N-dimethyl-*p*-phenylenediamine. At times small sheets of ductal epithelium also grew out of the fragments. These sheets were evidently of the cholangioma type since they exhibited the same appearance and same resistivity as the outgrowths of the cholangioma cultures. The toxic effect on the glandular epithelium of the normal liver was evidenced as early as 6 hours after exposure by a rounding-up and separation of the cells, although complete disintegration did not occur until about 24 hours later. A detailed observation of a large number of cultures suggested that the normal glandular epithelium is slightly more susceptible than the glandular epithelium of the tumor. However, the differences are so slight that they cannot be considered significant.

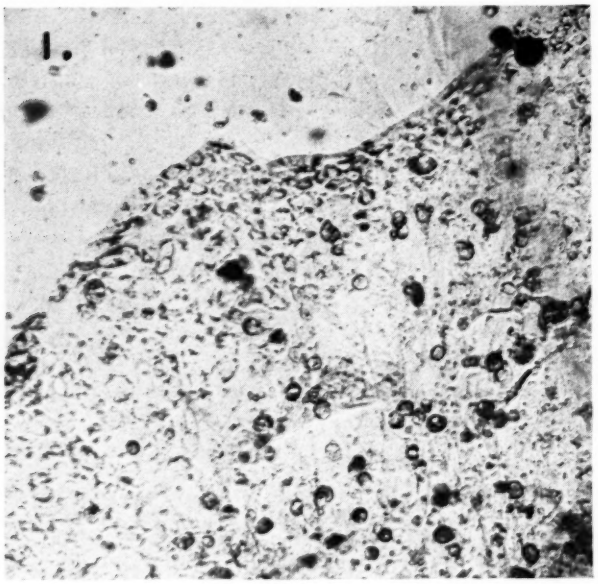
The appearance of intracellular fat-like drops in normal ductal epithelium exposed to 0.001 *M* N,N-dimethyl-*p*-phenylenediamine and none in the cholangiomas may be of real significance. However, the fat drops do not interfere with growth. It is possible that their presence may be the result of an intracellular detoxification mechanism. No attempt has been made to establish the identity of these inclusions with those described by Brues and Jackson (1), who reported that various primary amines produced an intense vacuolization of the cytoplasm of various non-malignant tumor cells cultured *in vitro*.

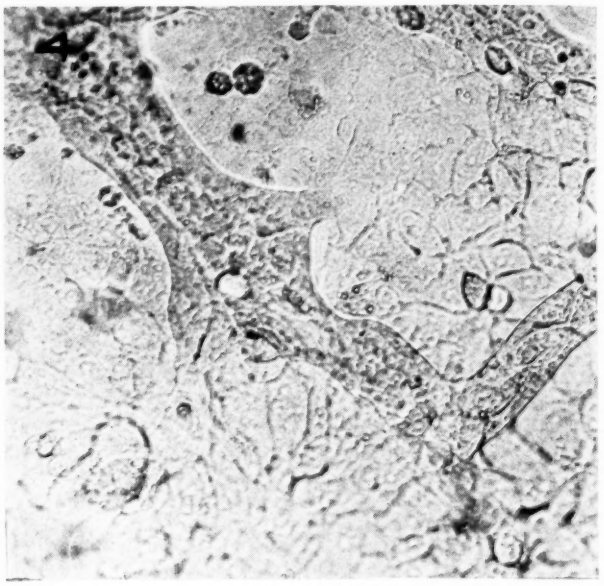
The wandering cells in the liver cultures consisted of a few fibroblasts and some Kupffer cells. These cellular elements differ from epithelial tissue in giving no evidence of specificity in their reactions to N,N-dimethyl-*p*-phenylenediamine. The Kupffer cells and fibroblasts appear to be equally susceptible in both tumor and normal cultures. Both types are more susceptible than ductal epithelium irrespective of whether they are normal or neoplastic.

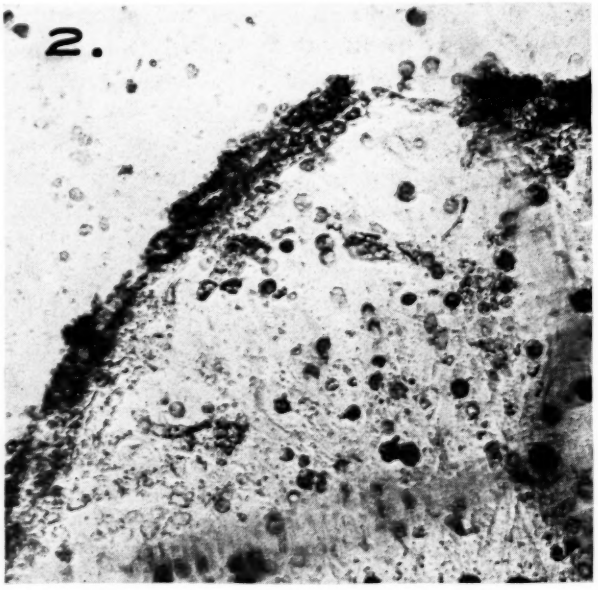
In addition to normal liver and liver tumors, the following tissues were studied: embryonic liver; regenerating normal liver; partially protected liver (*p*-dimethylaminoazobenzene + protective diet feeding) and the normal appearing portions of tumor-bearing livers—all from the rat; and mouse liver carcinoma C954.[2] In every case, the ductal epithelium survived in 0.001 *M* N,N-dimethyl-*p*-phenylenediamine, while the glandular epithelium was destroyed.

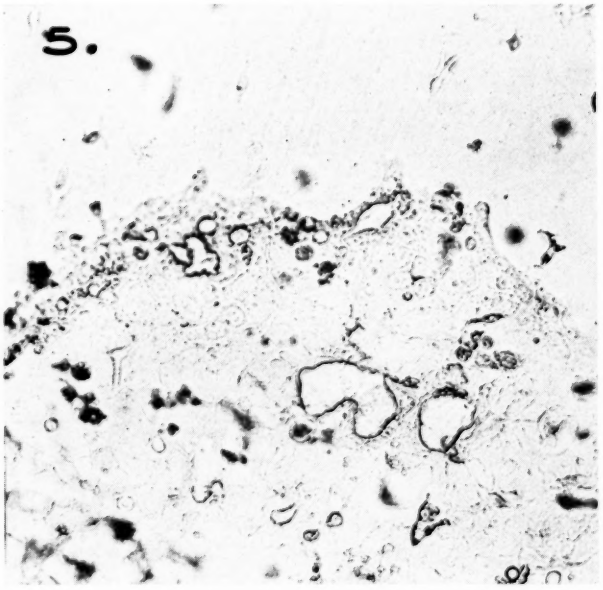
In higher concentrations there were indications of differences in susceptibility and in survival time of some of the tissues. The glandular portions of protected livers and the uninvolved portions of tumor-bearing livers survived a few hours longer than normal glandular epithelium. Similar shades of difference appeared between parenchymatous carcinomas and normal glandular epithelium. These differences were not sufficient within the scope of this experimentation to be emphasized.

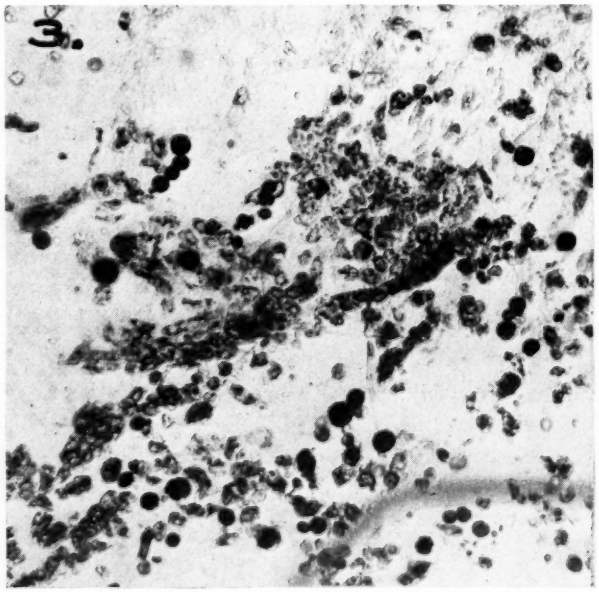
[2] Obtained from Dr. C. C. Little, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

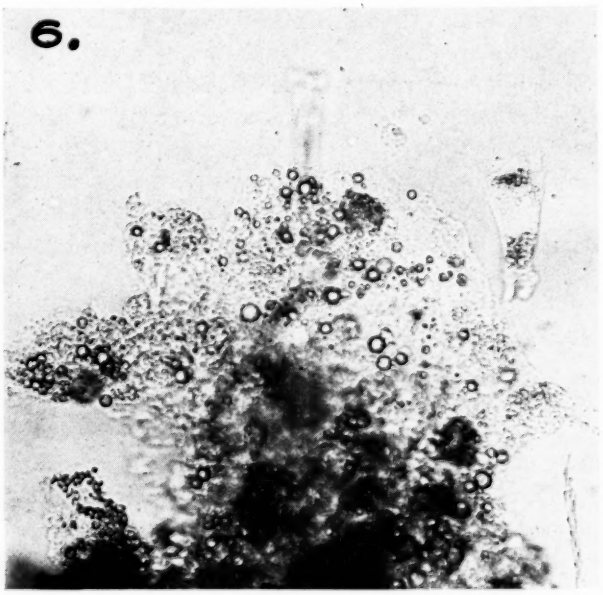












FIGS. 1-6

The more significant of the experimental results are summarized in Table I. Figs. 1 to 6 illustrate certain features.

The conclusions from these studies are that the glandular cells from normal adult and from tumor livers are almost equally susceptible to N,N-dimethyl-*p*-phenylenediamine, the sheets from a normal liver succumbing a few hours earlier than those from neoplasms. The ductal epithelium from both normal and tumor livers is resistant and continues to grow although cytoplasmic fat drops develop in the cells of normal liver cultures.

TABLE I: TYPICAL EFFECTS PRODUCED BY N,N-DIMETHYL-*p*-PHENYLENEDIAMINE (0.001 *M*) ON OUTGROWING EPITHELIAL SHEETS (RAT LIVER)

Length of exposure, hours	Glandular		Duct cell	
	Normal	Parenchymatous carcinoma	Normal	Cholangioma
3–4	Incipient retraction of sheets. Marginal cells rounding up (Fig. 1)	No effect	No effect	
6–7	Separation of cells more pronounced (Fig. 2)	Incipient retraction of sheets. Marginal cells rounding up	Occasional rounding up of cells on margin of sheets	
24	Sheets disorganized by complete separation of their cells. Cells beginning to disintegrate (Fig. 3)	Separation of cells more pronounced	Some marginal cells still rounded. Sheets intact. Some cells contain fat droplets (Fig. 6)	Sheets recovering and growing (Figs. 4 and 5)
48	Cells completely disintegrated	Sheets disorganized by complete separation of their cells. Cells beginning to disintegrate	Sheets recovering, forming cysts and aberrant ducts. Fat drops persist	Complete recovery. Outgrowing sheets form cysts and aberrant ducts (Figs. 4 and 5)

THE EFFECTS OF N,N-DIMETHYL-*p*-PHENYLENEDIAMINE COMBINED WITH REDUCING AGENTS

N,N-Dimethyl-*p*-phenylenediamine is an example of the aromatic paradiamines or Wurster's salts (compounds 2 and 3, Fig. 7). The oxidation-reduction characteristics of these compounds have been investigated by Michaelis, Schubert, and Granick (8). N,N-Dimethyl-*p*-phenylenediamine, in common with other paradiamines, can be oxidized to the free radical (loss of one electron = one-step oxidation), the latter frequently forming dimeres. The free radical (compound 8, Fig. 7) can be further oxidized to the diimine by losing one electron (compound 9, Fig. 7). Ultimately, N,N-dimethyl-*p*-phenylenediamine may also yield quinone together with methyl- or dimethylamine and formaldehyde (5).

We have found that oxidized solutions, colored deep red to blue, are nontoxic even to glandular epithelium. The toxic nature of N,N-dimethyl-*p*-phenylenediamine in Tyrode solutions is confined to the incipient pink solutions, presumably the free radical or its dimere. In the latter condition, the dimethyl compound at concentrations of 0.001 *M* is toxic to glandular epithelium and nontoxic to ductal epithelium. Kensler, Dexter, and Rhoads (5) have previously stated that the inhibition of the diphosphopyridine nucleotide system by N,N-dimethyl-*p*-phenylenediamine or by *p*-phenylenediamine is caused by the formation of an intermediary oxidation product.

Furthermore, we have found that the toxic action of 0.001 *M* N,N-dimethyl-*p*-phenylenediamine on glandular epithelium can be modified by the presence of a suitable reducing agent. The results are summarized in Table II.

Concentrations of sodium sulfite ranging from 0.0025 to 0.005 *M* protect glandular epithelium against the destructive action of 0.001 *M* N,N-dimethyl-*p*-phenylenediamine. Sodium sulfite suppresses the autoxidation of the dimethyl compound, since these solu-

DESCRIPTION OF FIGURES 1 TO 6

FIG. 1.—Growing sheet of glandular epithelium from normal adult rat liver. Mag. about × 300.

FIG. 2.—Same sheet after 6 hours' exposure to 0.001 *M* solution of N,N-dimethyl-*p*-phenylenediamine. Note beginning of disintegration with clumping and rounding of cells, especially on periphery.

FIG. 3.—Same sheet 24 hours later. Completely disintegrated.

FIGS. 4 and 5.—Growing sheets of ductal epithelium from rat tumor induced by *p*-dimethylaminoazobenzene. These were treated similarly to those of Fig. 1 but with no effect. Note cyst formation in Fig. 5 and duct-like arrangement of cells in Fig. 4.

FIG. 6.—Growing sheet of ductal epithelium from normal rat liver showing, by formation of fat droplets, the effect of 24 hours' exposure to 0.001 *M* N,N-dimethyl-*p*-phenylenediamine solution. Culture otherwise healthy and active.

tions remain colorless for many hours. On the other hand, unprotected solutions begin to oxidize within a few minutes after their preparation. One may infer that the epithelium is relatively impermeable to the reduced molecule (diamine) of N,N-dimethyl-*p*-phenylenediamine. The penetrating form appears to be either the free radical, its dimere, or the diimine (6).

Accordingly, any agent that either prevents the formation of the free radical of N,N-dimethyl-*p*-phenylenediamine or accelerates its oxidation beyond the diimine will lower the toxic action of the dimethyl compound. Systems containing sodium sulfite at concentrations of 0.0005 to 0.001 *M* are almost as toxic as those containing N,N-dimethyl-*p*-phenylenediamine alone. Cysteine hydrochloride at concentrations up to 0.005 *M* does not protect glandular epithelium cultured *in vitro* against the dimethyl compound. It is of interest to note that cysteine, 3 mgm. per 4 ml., did not significantly suppress the inhibition of diphosphopyridine nucleotide by the dimethyl compound (5), although Potter (11) later reported that cysteine at 0.001 *M* suppresses the inhibition of urease by the same compound.

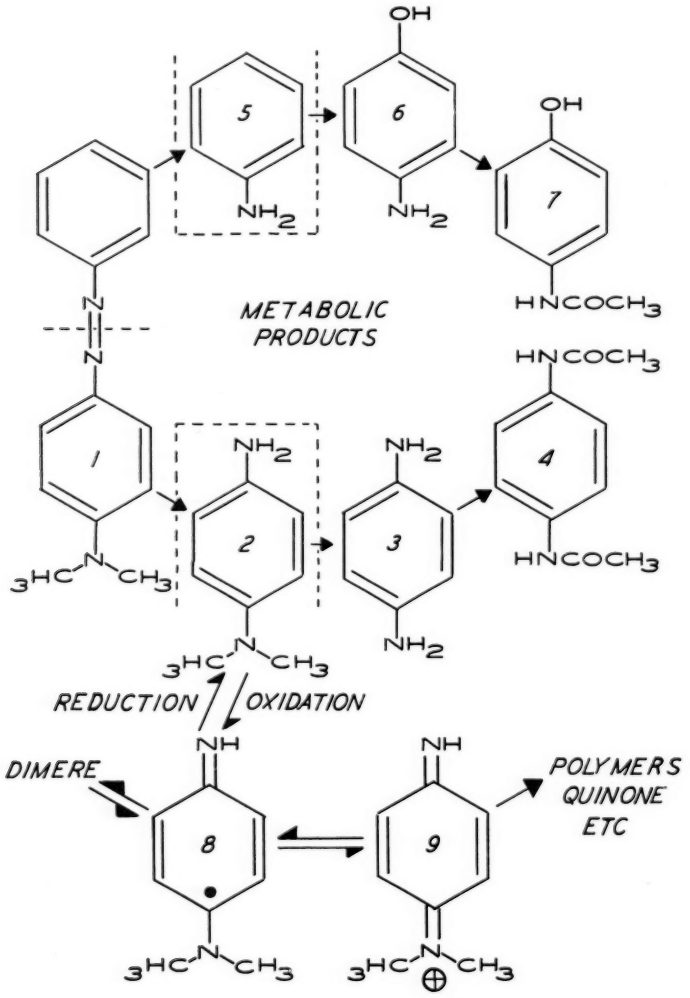


FIG. 7.—Metabolic products of *p*-dimethylaminoazobenzene according to Stevenson, Dobriner, and Rhoads (12), and oxidation of N,N-dimethyl-*p*-phenylenediamine according to Kensler, Dexter, and Rhoads (5). The compounds are: (*1*) *p*-dimethylaminoazobenzene, (*2*) N,N-dimethyl-*p*-phenylenediamine, (*3*) *p*-phenylenediamine, (*4*) N,N′-diacetyl-*p*-phenylenediamine, (*5*) aniline, (*6*) *p*-aminophenol, (*7*) N-acetyl-*p*-aminophenol, (*8*) free radical of *2*, and (*9*) diimine of *2*. Compounds *2* and *5*, segregated by broken lines, are possible intermediate products of *1*.

DISCUSSION

One of the obvious requirements for successful chemical carcinogenesis is that the cells must remain viable in the presence of the carcinogen as well as its split or possible metabolic products. The first probable split products of *p*-dimethylaminoazobenzene, *in vivo,* are aniline and N,N-dimethyl-*p*-phenylenediamine. Derivatives of these two substances have been isolated in the urine of rats to which *p*-dimethylaminoazobenzene has been administered (12).

Evidence is presented in our report to show that the epithelial cells of cholangiomas are resistant to the action of N,N-dimethyl-*p*-phenylenediamine as

shown by their ability to grow in concentrations that kill the glandular epithelium obtained from normal livers or from parenchymatous carcinomas. The sensitivity of glandular epithelium to the dimethyl compound may be the basis for Orr's (9) belief that the primary action of *p*-dimethylaminoazobenzene is destructive to liver parenchyma.

Cells growing from normal ductal epithelium show considerable resistance to N,N-dimethyl-*p*-phenylenediamine, and this fact is highly significant. Such resistance to an agent lethal for the glandular epithelial cells suggests that the duct cells are more apt to survive and eventually become modified by *p*-dimethylaminoazobenzene to form carcinomatous growths.

TABLE II: DETOXIFICATION OF N,N-DIMETHYL-*p*-PHENYLENEDIAMINE (0.001 *M*) BY SODIUM SULFITE IN CULTURES OF NORMAL RAT LIVER AND LIVER TUMORS, EXPOSED FOR 48 HOURS

Tissue	Concentration of sodium sulfite, *M*	Effects on epithelium
Normal rat liver (1636)	0.0	Glandular epithelium destroyed; ductal epithelium growing, fat drops in cells
	0.005	No effect; both glandular and ductal epithelia growing
Mixed liver tumor (1660)	0.0	Glandular epithelium destroyed; ductal epithelium in good condition
	0.001	Glandular epithelium disintegrated; ductal epithelium vacuolated but not destroyed
	0.0025	Glandular epithelium in good condition; ductal epithelium in excellent condition, like controls
Cholangioma (1630)	0.0	Epithelium in good condition
	0.005	No effect; epithelium growing

Orr and Stickland (10) pointed out that two main types of carcinomas in rat livers are produced by the action of *p*-dimethylaminoazobenzene. One is derived from the liver parenchyma, giving rise to the parenchymatous carcinoma type. The other originates from bile duct epithelium to produce the cholangioma type.

Orr (9) observed that 26 of 56 rats produced either bile duct carcinomas or cystadenomas. Altogether, 51 of the 56 rats produced tumors that involved bile duct epithelium either alone or in combination with parenchymal epithelium (Orr's liver cell epithelium), while only 5 of the 56 rats developed growths that involved exclusively the parenchymal epithelium.

On the other hand, Edwards and White (3) concluded that both hepatomas (our parenchymatous carcinoma) and adenocarcinomas (cholangioma type) develop from one kind of cell; *i. e.,* the hepatic parenchymal cell. The difference in response of glandular and ductal epithelium to N,N-dimethyl-*p*-phenylenediamine, however, favors the views of Orr and Stickland.

Fig. 7 shows the various metabolic derivatives of *p*-dimethylaminoazobenzene, after Stevenson, Dobriner, and Rhoads (12). In this diagram are also shown the stages in oxidation of N,N-dimethyl-*p*-phenylenediamine, after Kensler, Dexter, and Rhoads (5), which is the most toxic of the split products. The action of other split products of *p*-dimethylaminoazobenzene on liver tissues cultured *in vitro* is discussed in another report (6).

By assuming (a) that the action of N,N-dimethyl-*p*-phenylenediamine is the same *in vivo* as in tissue culture and (b) that this compound is one of the metabolic products of *p*-dimethylaminoazobenzene, one can explain why cholangiomas develop rather than parenchymatous carcinomas. The same mechanism also helps explain the large number of cirrhotic livers that frequently arise from the feeding of *p*-dimethylaminoazobenzene to rats.

It is assumed that, among other factors, if *p*-dimethylaminoazobenzene is to become carcinogenic it must be present in the liver above certain critical concentrations (at present not known). Therefore a sufficiently high breakdown rate of the parent substance (compound *1,* Fig. 7) would prevent reaching that concentration essential to induce neoplastic growths in the liver. In such cases, *p*-dimethylaminoazobenzene becomes rapidly converted to aniline and N,N-dimethyl-*p*-phenylenediamine (compounds 5 and *2,* Fig. 7). Miller, Miner, Rusch, and Baumann (7) are of the opinion that the function of dietary inhibitors of tumor production may be to hasten the breakdown of the *p*-dimethylaminoazobenzene molecule.

p-Dimethylaminoazobenzene must be maintained at the carcinogenic level, while N,N-dimethyl-*p*-phenylenediamine is quickly detoxified, to produce a parenchymatous carcinoma of the liver. The dimethyl compound can be rendered nontoxic: (a) by acetylation to the nontoxic diacetyl derivative (compound *4,* Fig. 7), or (b) by being maintained at the reduced (diamine) level. On the other hand, if both *p*-dimethylaminoazobenzene and N,N-dimethyl-*p*-phenylenediamine are present at higher concentration levels only cholangiomas may be formed. The former substance promotes carcinogenesis in the ductal epithelium while N,N-dimethyl-*p*-phenylenediamine destroys the glandular epithelium, thereby preventing these cells from becoming carcinomatous.

Most of the livers that we have obtained from rats fed on diets containing *p*-dimethylaminoazobenzene possess both normal and cirrhotic regions. In the

same livers, one may find cholangiomas and occasionally the gland cell tumors (10). The spotty nature of tumor development following the administration of *p*-dimethylaminoazobenzene suggests that the breakdown rates for this compound are not uniform throughout the liver and that there exist considerable variations in reducing potentials. The zones of the liver may be represented by four types: (a) unchanged or normal regions, (b) cirrhotic regions, (c) cholangiomas, or (d) parenchymatous carcinomas.

Obviously, in the unchanged region, both *p*-dimethylaminoazobenzene and the dimethyl compound must be maintained at low levels by the rapid conversion of the parent substance to the acetyl derivatives of aniline and of N,N-dimethyl-*p*-phenylenediamine or *p*-phenylenediamine (compounds *4* and *7*, Fig. 7). The maintenance of N,N-dimethyl-*p*-phenylenediamine at a high concentration, > 0.005 *M*, would result in the destruction of both ductal and glandular epithelia, eventually producing cirrhosis. This requires a high production of the dimethyl compound, combined with a negligible formation of acetyl derivatives, in a medium that promotes oxidation from the diamine to the free radical or diimine levels.

Cholangiomas can be formed in those portions of the liver that maintain *p*-dimethylaminoazobenzene at a carcinogenic level and also split this compound so that N,N-dimethyl-*p*-phenylenediamine is present at concentrations high enough to destroy glandular epithelium, > 0.0001 *M*, without destroying the ductal epithelium, < 0.004 *M*. A concentration of 0.001 *M* does this in tissue culture. The medium must be favorable for the oxidation of the dimethyl compound at least to the intermediate stages. On the other hand, parenchymatous carcinomas can develop in those portions of the liver where the carcinogen is maintained at a high level, while the dimethyl compound must either be transformed to its diacetyl derivative or the environment must be such that the diamine is kept reduced and thereby the toxic intermediates can be present in subtoxic concentrations only.

SUMMARY

Tissue cultures were made from the following rat tissues: normal adult livers, liver tumors induced by *p*-dimethylaminoazobenzene, uninvolved portions of tumor-bearing livers, partially protected livers (*p*-dimethylaminoazobenzene + protective diet), normal regenerating livers, and embryonic livers.

It was determined that normal glandular epithelium of the rat liver, grown in tissue culture, is sensitive to N,N-dimethyl-*p*-phenylenediamine in concentrations as low as 0.0001 *M* under autoxidizable conditions, while equivalent effects are produced on ductal epithelium with concentrations of 0.002 *M* or higher. The resistance of ductal epithelium from normal liver is slightly less than that obtained from tumors. Cytoplasmic fat drops develop in the cells but do not inhibit growth. Glandular liver epithelium, whether normal or from a parenchymatous carcinoma, is sensitive to the action of N,N-dimethyl-*p*-phenylenediamine, the only difference being that the cells of neoplastic origin survive from 12 to 18 hours longer.

The toxic action of N,N-dimethyl-*p*-phenylenediamine, 0.001 *M*, on glandular epithelium (normal or from a tumor) can be suppressed by sodium sulfite. Concentrations of sodium sulfite ranging from 0.001 to 0.005 *M* retard or prevent the oxidation of N,N-dimethyl-*p*-phenylenediamine and in the reduced state this compound is nontoxic. Completely oxidized N,N-dimethyl-*p*-phenylenediamine is also nontoxic. The toxicity of the dimethyl compound is probably confined to the intermediate stages in its oxidation.

The resistance of normal ductal epithelium to the dimethyl compound explains the preponderance of cholangioma production in livers following the feeding to rats of *p*-dimethylaminoazobenzene plus nonprotective diets. The occasional presence of gland cell tumors is believed to be the result either of the detoxification of N,N-dimethyl-*p*-phenylenediamine in the liver tissue, to its diacetyl derivatives, or of its maintenance in the reduced, nontoxic state. Liver glandular epithelium can survive only when the oxidizable concentration of this compound is low, < 0.0001 *M*, or when in the form of a diamine. High concentrations, > 0.005 *M*, under autoxidizable conditions are probably responsible for cirrhotic conditions in the liver following the feeding to rats of *p*-dimethylaminoazobenzene.

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X. The Effects in Tissue Culture of Some Split Products of *p*-Dimethylaminoazobenzene on Rat Liver Tumors*

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Stevenson, Dobriner, and Rhoads (5) reported that *p*-dimethylaminoazobenzene when fed to rats is decomposed and metabolized to several simpler compounds. One of these split products, N,N-dimethyl-*p*-phenylenediamine, has been found by us (1) to be toxic to the glandular epithelium of the rat liver at concentrations of 0.0001 *M*, while equivalent effects on the ductal epithelium were produced only with concentrations of 0.002 to 0.004 *M*. The other compounds that may be derived from *p*-dimethylaminoazobenzene or that have been recovered from the urine of rats fed it are the following: aniline, *p*-aminophenol, N-acetyl-*p*-aminophenol, *p*-phenylenediamine, and N,N'-diacetyl-*p*-phenylenediamine (5).

The tissue culture studies reported in this paper are on the effects of the nonacetylated split products mentioned above on normal rat liver and on liver tumors. The tissue cultures were prepared as described in the preceding report (1), and when good growths were established comparable cultures were selected and exposed to a medium containing one of the split products. This was done by replacing a portion of the serum component in the medium with Tyrode solution containing the split product, the final concentration of the experimental compound being given below.

Neither *aniline hydrochloride, aniline sulfate,* nor *p-aminophenol hydrochloride* at concentrations of 0.001 *M* produces effects on wandering cells or on gland or duct epithelia, regardless of their source, *i. e.,* normal or neoplastic. This contrasts with our previous finding (1) that N,N-dimethyl-*p*-phenylenediamine at a concentration of 0.001 *M* destroys glandular epithelium but is relatively harmless to ductal epithelium.

p-Phenylenediamine or *p-phenylenediamine hydrochloride,* at a concentration of 0.001 *M,* produces either one or two general reactions. One concerns the toxicity of *p*-phenylenediamine to some of the wandering cells and the epithelium growing from the explant. Some destruction of the latter was observed, particularly along the margins of the outgrowing epithelial sheets. The cells were disintegrated and became heavily stained a deep brown or a reddish brown. The resulting colored precipitate is presumably an insoluble complex between the oxidation products of *p*-phenylenediamine and the cytoplasmic residue.

The other, a most striking reaction, is the following: Within 24 hours after exposure of normal liver or liver tumor cultures to *p*-phenylenediamine, opaque, blue-black to black, aciform crystals appeared in the surviving epithelial and wandering cells. The crystals were seen in the cytoplasm, never in nuclei or the medium, lying singly or in compact groups forming bundles, rosettes, or fans (Figs. 1 and 2); occasionally their tips seemed to protrude through the cellular surface. All the cells, epithelial or wandering, that contained crystals were in good condition.

After 72 hours' exposure, many epithelial cells with the onset of disintegration had lost their crystals, and none were observed in dying or dead cells, nor were they liberated as such into the medium on the breakdown of the cell. Orange colored vacuoles gradually developed in the wandering cells, and the crystals disappeared before these cells disintegrated. At this time, only the crystal-containing cells were alive.

The epithelial cells that contained crystals were still alive after 96 hours' exposure. Some of the living fibroblasts enclosed a few crystals together with numerous grayish blue cytoplasmic granules that imparted a bluish tint to these cells and stood out in contrast to the colorless nuclei. The macrophages had become progressively moribund with the disappearance of crystals and their replacement by reddish brown granules. These cells soon disintegrated.

The development of crystals in the cytoplasm of surviving cells is apparently an intracellular mechanism for detoxifying the diamine. This follows from

* In cooperation with Dr. C. P. Rhoads and Mr. C. J. Kensler, Memorial Hospital, New York, N. Y., and with Dr. G. H. A. Clowes, The Lilly Research Laboratories, Indianapolis. This investigation was aided by The International Cancer Research Foundation and The Lilly Research Laboratories of Eli Lilly and Company.

numerous observations that *p*-phenylenediamine kills those cells which do not, for some reason, convert the penetrating substance into the insoluble crystalline product.

The precise nature of the crystals is unknown, although these may consist of polymeric derivatives of oxidized *p*-phenylenediamine. Many such polymers are known in the field of dye chemistry (4). In this connection, several oxidizing agents were added to test tubes containing a solution of *p*-phenylenediamine. Almost immediately after the oxidant had been added a precipitate would form associated with a change in color of the solution. With hydrogen peroxide, the reddish brown precipitate was an amorphous, jelly-like mass. With potassium ferricyanide, the dark blue precipitate consisted of crystals of various shapes including the aciform type. When a drop of solution from the latter preparation was evaporated, aciform and other crystalline patterns of microscopic dimensions would develop. The dry crystals dissolved on addition of water. It is entirely possible that the crystals appeared in the cytoplasm because the oxidized product of *p*-phenylenediamine had a low solubility in cytoplasm. Likewise, the failure to find crystals in the medium following the disintegration of cells that produced them may have been the result of an apparent higher solubility of the oxidized crystalline product in the culture medium.

Kensler, Dexter, and Rhoads (2) found that cysteine, 3 mgm. per 4 ml., suppresses the inhibition of the diphosphopyridine nucleotide system by *p*-phenylenediamine. Potter (3) reported that cysteine, 0.001 *M*, likewise suppresses the inhibition of urease by *p*-phenylenediamine. On the other hand, cysteine hydrochloride in concentrations as high as 0.005 *M* does not detoxify *p*-phenylenediamine to permit the survival of liver cells grown in tissue culture. Following 24 hours' exposure to cysteine (0.005 *M*) + *p*-phenylenediamine (0.001 *M*) glandular and ductal epithelia were dead and stained a deep reddish brown. The fibroblasts, however, had begun to develop crystals and were therefore able to survive.

The observations just cited may be significant. They might indicate that, in liver cells grown in tissue culture, systems other than diphosphopyridine nucleotide or urease, or other sulfhydryl-containing enzymes, are involved, an idea previously suggested by Potter. Another interpretation is that cysteine in the tissue culture medium, pH = 7.6, *inter alia,* enhances the penetrability of *p*-phenylenediamine into liver cells. This matter is considered in greater detail below.

The addition of 0.001 *M* sodium sulfite, an agent commonly used for preventing autoxidation, to the culture medium inactivated *p*-phenylenediamine by preventing both the destruction of epithelial cells and the formation of crystals in the cytoplasm. None of

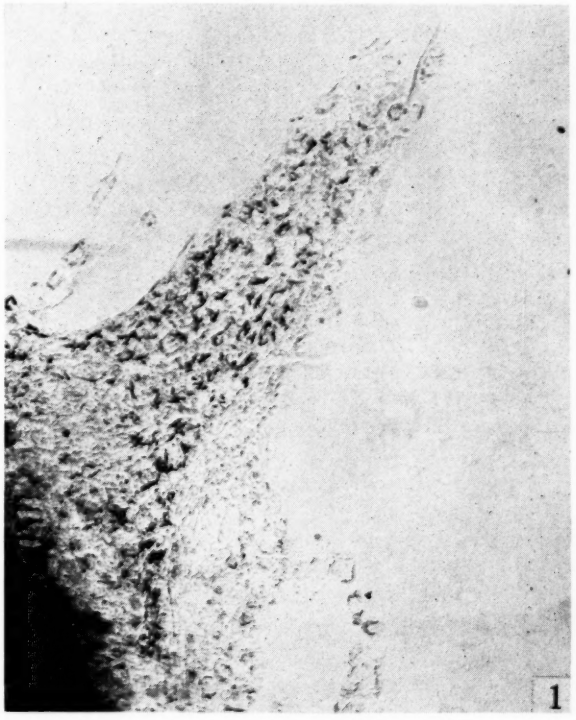

FIG. 1.—Low power view of growing epithelial sheet showing the distribution of crystals in the cells. Mag. × 100.

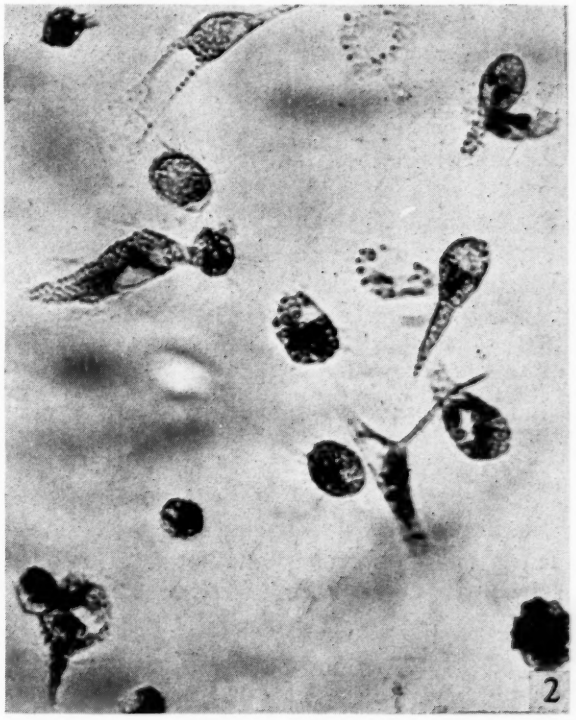

FIG. 2.—High power view of living Kupffer cells showing bundles and rosettes of crystals in the cytoplasm. The nuclei are clear and colorless. Mag. × 450.

the cells showed any kind of crystal development during the first 48 hours' exposure and still the cultures were in good condition and compared favorably with the controls. After 72 hours' exposure crystals began to develop in some of the wandering cells. All cultures containing sodium sulfite were in better condition than those grown in media with *p*-phenylenediamine alone.

The oxidation of *p*-phenylenediamine is retarded by sodium sulfite. The survival of cells without crystal formation indicates that the reduced *p*-phenylenediamine (diamine stage) penetrates very slowly, if at all; the molecules presumably penetrate the cells in the form of the free radical, its dimere, or the diimine. The oxidation of the diamine to free radicals and beyond is retarded or inhibited by sodium sulfite, depending on the concentration of the latter.

Similar results were previously obtained with culture media containing sodium sulfite, > 0.0025 *M*, and N,N-dimethyl-*p*-phenylenediamine (1). Although the latter compound does not form intracellular crystals, its penetration into glandular and ductal epithelia is inferred from its toxic action. The toxic effect can be completely blocked by sodium sulfite. Apparently, the penetrating form of N,N-dimethyl-*p*-phenylenediamine is likewise the free radical, its dimere, or the diimine.

SUMMARY

Several split products of *p*-dimethylaminoazobenzene were tested on normal liver and rat liver tumors grown in tissue culture. Aniline hydrochloride or aniline sulfate, also *p*-aminophenol hydrochloride at concentrations of 0.001 *M*, were nontoxic to both glandular and ductal epithelia. Either *p*-phenylenediamine or *p*-phenylenediamine hydrochloride, under autoxidizable conditions, at 0.001 *M* was toxic to all cells except those that form intracellular crystals (possible oxidation polymers of the diamine). Sodium sulfite blocked intracellular crystal formation and, in such cases, no toxicity of *p*-phenylenediamine could be demonstrated.

None of the split products mentioned above was found to be so toxic as N,N-dimethyl-*p*-phenylenediamine, which destroyed normal or neoplastic glandular-cell epithelium at concentrations of 0.001 *M* (1). On the other hand, ductal epithelium (normal or neoplastic) at this concentration survived and grew.

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XI. The Effects in Tissue Cultures of N,N,N′,N′-Tetramethyl-*o*-Phenylenediamine and Other Compounds on Malignant Lymph Nodes*

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These experiments were conducted on the basis of recent unpublished findings by Kensler that the respiration of malignant lymphoid tissue is inhibited by tetramethyl-*o*-phenylenediamine while that of its normal counterpart is modified only slightly if at all. The investigations described in this report, which is the third of a series of viability experiments (1, 2), were planned to determine the effect of these and other compounds on the viability of lymphocytes from a variety of lymphoid tissues grown in tissue culture. The cultures of human material were of normal lymph nodes, lymphosarcoma, lymphatic leukemia, and nodes of Hodgkin's disease. Cultures were made also of normal rat lymph nodes and a transplantable rat lymphosarcoma (3). The studies reported are based on over 1,000 cultures, totalling about 3,000 fragments of human material and about 400 cultures of rat tissues.

For the controls the tissue culture medium consisted of fowl blood plasma, 1 part; homologous serum, 3 parts; chick embryonic extract, 1 part.

Immediately after explantation the cultures frequently showed an appreciable halo of cells about the explant, a condition to be distinguished from an active outwandering of cells. A few leukocytes might appear after 3 or 4 hours of incubation, while the lymphocytes began to migrate in increasing numbers after 8 to 10 hours.

The majority of the lymphocytes from normal nodes were of the small, round type, interspersed with a few of the intermediate and an occasional example of the large type. Cultures of the neoplastic nodes presented the typical appearance of lymphocytic outgrowths, except for the presence of considerable cellular debris, a greater variety of shapes and sizes of the outwandering cells, and frequency of the large type of lymphocytes.

In the majority of cases, suitable outgrowths for experimentation developed after 48 hours of incubation, while some attained sufficient proportions at 24 hours. Others were 24 to 48 hour growths from subcultured explants of fragments started a week or two earlier.

The compound to be tested was introduced into a growing culture by adding a medium in which the serum had been replaced by Tyrode solution or serum, containing a given concentration of the compound. The figures given below are those of the final concentrations.

The compounds employed were sodium malonate, hydroxyquinoline, rotenone, N,N-dimethyl-*p*-phenylenediamine, N,N,N′,N′-tetramethyl-*o*-phenylenediamine, and N,N,N′,N′-tetraethyl-*o*-phenylenediamine.

Sodium malonate was tested in concentrations from 0.01 to 0.05 *M*. The concentration of 0.05 *M* was found to be toxic in a few hours to all types of cells. It showed no differential action between lymphocytes and other wandering cells whether from normal or neoplastic tissues, including nodes from Hodgkin's disease. A concentration of 0.03 *M* had no toxic action during 48 hours of exposure.

8-Hydroxyquinoline, in a concentration of 0.2 saturation in serum, likewise showed a general toxic effect within 24 hours on cells of both normal and neoplastic origin but to a less pronounced degree. The effect on Hodgkin's nodes was not tested. Weaker concentrations (0.1 and 0.05 saturated) were nontoxic throughout.

Rotenone, in a concentration of 0.2 saturation in serum, was also found to be nonspecific in its toxic effect, which became evident only after 48 to 72 hours. A concentration of 0.1 saturation was definitely less effective but still nonspecific. An exception appeared to be the lymph nodes of Hodgkin's disease, the lymphocytes of which were slightly more resistant than those from normal nodes or from lymphosarcoma.

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N,N-Dimethyl-p-phenylenediamine, in a concentration of 0.001 *M,* was toxic to the lymphocytes but not to the macrophages and fibroblasts in cultures of all types of tissues used. A large proportion of the lymphocytes underwent complete degeneration within 5 to 6 hours, while the macrophages and fibroblasts were only slightly, if at all, affected. The macrophages tended to round up and become inactive but a few hours later recovered completely, although they developed yellow vacuoles which eventually became pink. The fibroblasts remained normal throughout. The fat drops of adipose cells acquired a pronounced and persistent red-orange color. The same coloration was evident in extracellular fat drops of older cultures. At a concentration of 0.004 *M* all the cells, including fibroblasts and macrophages, disintegrated within 24 hours.

Cells from the nodes of Hodgkin's disease reacted in a similar fashion except that the macrophages, Dorothy Reed cells, and fibroblasts became granular during a brief inactive period but subsequently recovered.

N,N,N',N'-Tetramethyl-o-phenylenediamine. — The results of exposure to this compound are summarized in Table I. The macrophages and fibroblasts in cultures of normal and malignant tissues were equally unaffected by exposure to concentrations up to and including 0.01 *M* of the compound. With time the cells gradually acquired yellow inclusion bodies. Occasionally, after exposures of about 48 hours to concentrations of 0.01 *M,* the macrophages developed pink vacuoles. The difference between the normal and neoplastic tissues appeared in the reactions of the lymphocytes.

For lymphocytes of normal lymph nodes the critical concentration was 0.0075 *M.* In this concentration some of the lymphocytes showed a retraction of their pseudopodia and an infrequent increase in granulation of the cytoplasm. These changes were temporary, the lymphocytes returning to their normal appearance within a few hours. Disintegration occurred only very rarely. A concentration of 0.005 *M* exerted no effect whatsoever.

For the lymphoid cells of the neoplastic tissues the critical concentration in general was 0.001 *M,* lower than that for the normal tissues, which was 0.0075 *M.* The lymphosarcomas showed a slight variability in their reaction, the toxic level varying in different tumors between 0.001 and 0.0025 *M.* The toxic effect on the cells was first made evident by retraction of their pseudopodia. Granulation of the cytoplasm steadily increased until the final stage of complete disintegration was reached. All stages up to disintegration were reversible. In borderline concentrations short of the toxic dose, the lymphocytes frequently appeared unhealthy after a sojourn of 5 to 6 hours in the experimental medium. However, after 24 hours they had resumed their normal appearance and activity. In the higher toxic concentrations of 0.01 and 0.05 *M* the lymphocytes did not undergo disintegration but were killed by fixation within a few hours. The nuclei of these fixed cells frequently acquired a pink color.

TABLE I: EFFECT OF N,N,N',N'-TETRAMETHYL-*o*-PHENYLENEDIAMINE ON WANDERING CELLS IN CULTURES OF HUMAN LYMPHOID TISSUES

Molar concentrations	Macrophages, fibroblasts, normal and from neoplasms	Lymphoid cells from nodes of		
		Hodgkin's disease	Lymphosarcoma, lymphatic leukemia	Normal subject
0.05	Toxic	Toxic	Toxic	Toxic
0.01	Nontoxic	"	"	A few survivals
0.0075	"	"	"	Nontoxic
0.005	"	"	"	"
0.0025	"	A few survivals	"	"
0.001	"	Nontoxic	A few survivals	"

The sensitivity of lymphoid cells from the nodes of Hodgkin's disease was similar to that of the lymphosarcoma. In concentrations of 0.005 and 0.0075 *M* they were invariably destroyed, either by disintegration or by coagulation, the nuclei becoming pink. The Dorothy Reed cells were more resistant, resembling in this way the macrophages except for a tendency to become highly granular and vacuolated. In common with the macrophages and fibroblasts, the Dorothy Reed cells developed yellow inclusions after an exposure of 24 hours or longer.

The rat lymphosarcoma and normal lymph nodes grew excellently in the cultures. Their growth was more uniform than that of the human material and their sensitivity to the toxic compounds used was more consistent. This uniformity in growth and reaction, in contrast to that of the human lymphosarcomas, may be due to the controlled source of supply in the experimental rat.

N,N,N',N'-Tetraethyl-o-phenylenediamine was tested on cultures of human neoplastic nodes. Its action was similar except for its lesser toxicity, the critical toxic concentration being 0.005 *M,* or double that for the tetramethyl compound.

DISCUSSION AND SUMMARY

The compounds tested on normal and neoplastic lymphoid tissues may be divided into three groups, according to their action on the outgrowing cells of the cultures.

In the first were those the minimum effective concentration of which indiscriminately destroyed the cells. These were sodium malonate, 0.005 *M;* 8-hydroxyquinoline, 0.2 saturation in serum; and rotenone, 0.1 saturation in serum.

In the second group was N,N-dimethyl-*p*-phenylenediamine which, at a concentration of 0.001 *M,* destroyed all lymphoid cells irrespective of their source but did not affect the macrophages and fibroblasts. A concentration sufficient to destroy macrophages and fibroblasts was 0.004 *M*.

In the third group were N,N,N′,N′-tetramethyl-*o*-phenylenediamine and N,N,N′,N′-tetraethyl-*o*-phenylenediamine. Both resembled N,N-dimethyl-*p*-phenylenediamine in exerting a more drastic action on lymphocytes than on macrophages or fibroblasts. On the other hand, they were definitely more toxic to the lymphoid cells of the neoplastic than to those of the normal lymph nodes.

The critical toxic levels for lymphoid cells of the various cultured tissues to tetramethyl-*o*-phenylenediamine were found to be as follows:

Normal lymph nodes (human and rat)	0.0075 *M*
Hodgkin's disease	0.0025 *M*
Lymphosarcoma (human and rat)	0.001–0.0025 *M*
Leukemic node (human)	0.001 *M*

The closely related tetraethyl-*o*-phenylenediamine was tested only on human lymphosarcoma and leukemic nodes. It was found to have a similar specific toxicity for lymphocytes but with a potency of about half that of tetramethyl-*o*-phenylenediamine.

It is not possible from present data to decide upon the actual identity of the lymphoid cells of the normal and malignant tissues studied. The majority of the cells appearing in cultures of normal lymph nodes are of the small round cell type, but those of similar appearance in malignant nodes may not be precisely those of the normal nodes. This is suggested by greater variety in the shape of cells in malignant cultures and their tendency to form irregularly lobate instead of the characteristic relatively minute and narrow pseudopodia of the normal lymphocytes. They may be of an immature type, which raises the possibility that immaturity *per se* is the causative factor in their excessive sensitivity. However, the over-all difference in sensitivity of lymphoid cells from malignant and normal sources to tetraethyl- and tetramethyl-*o*-phenylenediamine is sufficiently pronounced to be significant. It supports the findings of Kensler in his respiration studies.

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The Effect of Pyridoxin and Other B Vitamins on the Production of Liver Cancer with *p*-Dimethylaminoazobenzene*†

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INTRODUCTION

It appears well established that the incidence of tumors resulting from the feeding of *p*-dimethylaminoazobenzene depends upon the character of the diet fed during the precancerous period. Factors reported to retard tumor development include liver (19), yeast (18), grain (16), the combination of casein and riboflavin (11), the combination of protein and B vitamins (16), and the combination of cystine and choline (6). Biotin (5) and cystine (25) are reported to be cocarcinogenic for this type of tumor. As might be expected, the cocarcinogenic and the anticarcinogenic effects observed depend upon the character of the basal rations fed. Casein and riboflavin are particularly anticarcinogenic when the basal diet consists of rice and carrot, or when the source of the vitamin B complex is a rice bran extract such as vitab (16), which contains all the B vitamins in abundance except riboflavin. Thus the effectiveness of the riboflavin could be essentially fortuitous, depending upon the specific deficiency in the crude materials employed in the basal ration. The effect of the other B vitamins in the genesis of hepatic tumors is still unknown. Some effect might be inferred, however, from the observation that the split products of the carcinogenic azo dyes inhibit certain enzymes that depend upon nicotinamide coenzymes or upon thiamin pyrophosphate for their activity (10,12).

The present study represents an attempt to determine the effect of each of the B vitamins in the production of hepatic tumors due to *p*-dimethylaminoazobenzene. This, of necessity, required the feeding of highly purified synthetic diets. As in our previous studies (16), young, mature albino rats 150 to 200 gm. in weight were fed 0.06 per cent of *p*-dimethylaminoazobenzene for a period of 120 days. The animals were kept in groups of 8 to 15 in screen-bottomed cages, and food and water were given ad libitum. The rations were mixed in amounts sufficient for 2 to 4 weeks and stored in the refrigerator. After 120 days the livers were examined by laparotomy and the animals continued for an additional 2 months on the same diet as before, but minus the azo dye.

Relative difficulty in the production of liver tumors in rats on synthetic diets.—Several workers have noted a decreased tendency toward tumor formation in animals maintained on synthetic diets as contrasted with nutritionally poorer diets consisting of crude materials. György, Poling, and Goldblatt (6) reported "cirrhosis, atypical nodular proliferation of bile ducts, adenocarcinoma, malignant hepatoma" in 80 to 100 per cent of their animals on a rice-carrot diet containing *p*-dimethylaminoazobenzene and crystalline thiamin, riboflavin, pyridoxin, and pantothenic acid, although no tumors were observed in rats fed a synthetic diet containing the azo dye. Du Vigneaud and his coworkers (5) observed that the addition of biotin definitely increased tumor production, but that in the absence of added biotin no tumors developed in 5 rats receiving a synthetic ration containing 0.1 per cent of *p*-dimethylaminoazobenzene. Antopol and Unna (1) fed 0.02 per cent of the azo dye in synthetic diets for periods up to 338 days and observed tumors in only 4 of 52 animals.

We have encountered a similar resistance toward tumor formation in animals fed synthetic diets of the following composition:

DIET A

Casein (vitamin-free)	180
Glucose	730
Corn oil	50
Salts mixture	40
p-Dimethylaminoazobenzene	0.6

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† Published with the permission of the Director of the Wisconsin Agricultural Experimental Station.

Each rat received one drop of halibut liver oil per week. Crystalline vitamin B supplements added per kilogram of ration were:

Thiamin hydrochloride	1.0 mgm.
Riboflavin	1.5 "
Pyridoxin hydrochloride	1.0 "
Calcium pantothenate	5.0 "
Nicotinic acid	1.0 "

These levels of the B vitamins are just sufficient for the growth of young rats (2, 3, 4, 21, 24). The vitamin mixture was demonstrated to be adequate for maintenance by feeding a somewhat similar ration free of the azo dye to adult rats for a period of 6 months. The maintenance ration, diet B, contained no nicotinic acid and the level of casein was reduced to 12 per cent. Other rats were maintained on similar rations from which various B vitamins were omitted (Table I). The control group of rats not only remained in good health for 6 months, but actually gained in weight. In the absence of thiamin, riboflavin, or pantothenate, however, the animals died after 2, 3, and 4 months respectively. When 2 μgm. of pyridoxin or less were fed daily, the animals survived for over 6 months, although they lost some weight. However, when the level of pantothenate was reduced from 5 to 1.5 mgm. per kg. of ration, the animals survived without any weight loss.

Nevertheless, the addition of 0.06 per cent of *p*-dimethylaminoazobenzene to the diet for 4 months failed to produce tumors. Numerous variations in the vitamin levels were introduced with each vitamin fed at both a very high and a very low level. The survival of the animals was relatively poor in all groups and on no diet did more than one tumor appear (Table II). This relatively low tumor incidence stands in sharp contrast to our previous results with cruder diets, when a tumor incidence of over 80 per cent was observed consistently (16).

The reason for the relatively low incidence of tumors on the synthetic diet is not yet clear. Part of the difference may have been due to the effect of the biotin present in the rice bran concentrate previously employed. It is also possible that the azo dye may have increased the requirement of the animals for any or for several of the B vitamins, and that the animals correspondingly were in too poor a nutritive

TABLE I: THE MAINTENANCE OF ADULT RATS ON VARIOUS SYNTHETIC DIETS IN THE ABSENCE OF *p*-DIMETHYLAMINOAZOBENZENE

Group	Variation in B vitamins from control level, mgm. per kg.	Average starting weight, gm.	Average weight at 6 months, gm.	Condition at 6 months
Control diet B		267	302	Healthy
Pantothenate	Reduced from 5.0 to 1.5	276	288	"
Riboflavin	Reduced from 1.5 to 0.5	258	222	"
Pyridoxin	Reduced from 1.0 to 0.2	262	217	"
No pyridoxin		242	220	"
No thiamin		247	135 *	Died after 2 months
No riboflavin		253	146 *	Died after 3 months
No pantothenate		254	133 *	Died after 4 months

* Weight at death.

TABLE II: INCIDENCE OF HEPATIC TUMORS IN RATS FED MODERATE AMOUNTS OF SYNTHETIC B VITAMINS

Group	Variation in B vitamins from control level, mgm. per kg. ration	Average food consumption, gm. per rat per day	Average starting weight, gm.	Average weight at 4 months, gm.	Survival * at 4 months	Tumors *	
						4 months	6 months
Control diet A	—	7.0	229	179	8/24	0/8	0/8
Low riboflavin	0.15	6.0	240	160	8/15	1/8	1/8
High riboflavin	15	6.5	210	155	11/14	0/11	0/11
Low thiamin	0.3	6.7	227	160	4/15	1/4	1/4
High thiamin	10	7.7	218	170	8/15	1/8	1/8
Low pyridoxin	0.1	5.0	215	159	4/15	0/4	0/4
High pyridoxin	10	6.7	222	169	8/15	1/8	1/8
Low pantothenate	0.5	6.1	227	141	6/15	0/8	0/8
High pantothenate	50	5.6	167	127	8/15	0/8	0/8
Low nicotinic acid	0.1	5.6	290	190	5/15	0/5	0/5
High nicotinic acid	10	6.2	245	165	8/15	0/8	0/8
12 per cent casein (as in diet A)	—	7.6	230	180	6/15	0/6	0/6

* Survival = Number of living over number at start.
Tumors = Number with tumors over number surviving at 4 months.

state for the effective production of hepatic tumors (25). Thus although borderline levels of riboflavin tend to favor tumor formation (11, 16), similar levels of all the B vitamins at once tend to suppress it.

Tumor production on synthetic diets.—Du Vigneaud and his coworkers (5) succeeded in producing tumors in 3 of 5 rats on a synthetic diet containing biotin in addition to their mixture of the regular crystalline B vitamins. We have been able to produce a high percentage of liver tumors in the absence of added biotin when the levels of the other B vitamins were increased significantly above those amounts needed for maintenance. The control ration, diet C, was patterned after our previously successful crude diet (16) and consisted of:

Casein (vitamin-free)	120
Glucose	790
Corn oil	50
Salts mixture	40
p-Dimethylaminoazobenzene	0.6

The crystalline B vitamins were incorporated in the ration at levels approximately equivalent to those in a diet containing 2 per cent of rice bran concentrate. Supplements added per kilogram of ration were:

Thiamin hydrochloride	3.0 mgm.
Riboflavin	2.0 "
Pyridoxin hydrochloride	2.5 "
Calcium pantothenate	7.0 "
Nicotinic acid	30.0 "
Choline chloride	30.0 "

One drop of halibut liver oil per week was given by dropper to each rat. In the absence of the azo dye this low protein diet was demonstrated to be adequate for the growth of young rats. A gain in weight from an average of 61 gm. to 213 gm. during 100 days of feeding was observed, an average daily increase of 1.5 gm.

In the carcinogenic diets other than the control the level of each of the B vitamins was lowered in turn, and nicotinic acid and choline were completely omitted from diets 6 and 7 respectively (Table III). One group of animals received 2 per cent of rice bran concentrate (diet 8, Table III) and another received 2 per cent of the concentrate in addition to a mixture of crystalline vitamins somewhat similar to that of the control ration (diet 9, Table III). The total amount of B vitamins in the latter diet was approximately equal to that in a ration containing 4 per cent of rice bran concentrate. The effect of rice protein was measured in diet 10, which contained 80 per cent of ground polished rice and 5 per cent of casein (total crude protein approximately 12 per cent). The rest of the diet consisted of glucose 6, salts 4, and corn oil 5 per cent respectively, and the B vitamins added were those of the control ration.

The animals on most of the diets survived well and some actually gained weight in the presence of the azo dye (Table IV). In the control group the tumor incidence was 33 per cent at 4 months and 66 per cent

Table III: The Vitamin B Content of the Diets Fed to Groups Listed in Tables IV and V

Group	Vitamins, mgm. per kg. of diet					
	Riboflavin	Thiamin hydrochloride	Pyridoxin hydrochloride	Calcium pantothenate	Nicotinic acid	Choline chloride
1. Control diet C	2.0	3.0	2.5	7.0	30.0	30.0
2. Low riboflavin	0.5	3.0	2.5	7.0	30.0	30.0
3. Low thiamin	2.0	0.6	2.5	7.0	30.0	30.0
4. Low pyridoxin	2.0	3.0	0.2	7.0	30.0	30.0
5. Low pantothenate	2.0	3.0	2.5	1.5	30.0	30.0
6. No nicotinic acid	2.0	3.0	2.5	7.0	0	30.0
7. No choline chloride	2.0	3.0	2.5	7.0	30.0	0
8. 2 per cent rice bran concentrate	0	0	0	0	0	0
9. 2 per cent rice bran concentrate plus vitamins	0.5	3.0	2.5	7.0	30.0	30.0
10. 80 per cent rice	2.0	3.0	2.5	7.0	30.0	30.0
11. 2 per cent bran concentrate plus high riboflavin	10.0	0	0	0	0	0
12. Control diet C	2.0	3.0	2.5	7.0	30.0	30.0
13. High thiamin	2.0	10.0	2.5	7.0	30.0	30.0
14. No pyridoxin	2.0	3.0	0	7.0	30.0	30.0
15. No pyridoxin High pyridoxin *	2.0 2.0	3.0 3.0	0 10.0 *	7.0 7.0	30.0 30.0	30.0 30.0
16. 18 per cent casein	2.0	3.0	2.5	7.0	30.0	30.0
17. 18 per cent casein High pyridoxin	2.0	3.0	10.0	7.0	30.0	30.0

* Pyridoxin was fed only after the 3rd month.

at 6 months. No pronounced effect on tumor incidence was noted when the content of thiamin was either greatly decreased or increased (diets 3 and 13, Table IV), nor did a reduction in the amounts of riboflavin, calcium pantothenate, or choline chloride (diets 2, 5, 7, Table IV) from the control level notably affect tumor production. The omission of nicotinic acid from the control ration appeared to increase the incidence of tumors somewhat (diet 6, Table IV). In the presence of 2 per cent of the rice bran concentrate the incidence of tumors was 100 per cent (diet 8, Table IV) as in our previous experiments (16), and the addition of 10 mgm. of riboflavin per kg. of ration completely prevented the appearance of tumors by 6 months (diet 11, Table IV).

Kensler and his coworkers (11) failed to observe much protection against tumor formation when riboflavin alone was added to a rice-carrot diet containing the azo dye. Presumably the limiting factor in this particular combination was protein. With crude diets we have observed a considerable lowering in tumor incidence when the level of casein in the diet was raised above 12 per cent (16). Where riboflavin was the limiting factor in our present experiments (diets 8 and 11, Table IV, and diets 1 and 3, Table V), an increase in this vitamin alone completely prevented tumor formation by 6 months. Furthermore, tumor incidence on synthetic diets containing 18 per cent of casein (diets 16 and 17, Table IV) was essentially the same as when 12 per cent of casein was fed (diets 1 and 12, Table IV).

As indicated in our previous work (16), protection at 6 months does not necessarily imply abolition of all subsequent tumor formation. We have occasionally noted some tumors developing as long as 7 months after the azo dye had been withdrawn from the diet.

TABLE IV: INCIDENCE OF HEPATIC TUMORS IN RATS FED HIGH AMOUNTS OF SYNTHETIC B VITAMINS

Group	Average starting weight, gm.	Weight at 4 months, gm.	Average food consumption, gm. per rat per day	Survival * at 4 months	Tumors *		Cirrhosis
					4 months	6 months	
1. Control diet C	190	167	6.5	12/14	4/12	8/12	Severe
2. Low riboflavin	194	144	6.2	11/14	2/11	6/11	Severe
3. Low thiamin	155	130	5.6	14/15	4/14	9/14	Moderate
4. Low pyridoxin	191	195	8.2	15/15	0/15	1/15	None or mild
5. Low pantothenate	197	223	10.0	14/14	4/14	7/14	Mild
6. No nicotinic acid	192	183	8.0	14/15	7/14	13/14	Severe
7. No choline	192	170	7.2	10/15	5/10	8/10	Severe
8. 2 per cent rice bran concentrate	192	153	7.7	8/12	3/8	8/8	Severe
9. 2 per cent rice bran concentrate plus vitamins	168	190	9.8	13/14	11/13	13/13	Mild
10. 80 per cent rice	219	283	11.6	10/14	5/10	6/10	Mild
11. 2 per cent bran concentrate plus high riboflavin	209	207	8.7	13/15	0/13	0/13	None or mild
12. Control diet C	161	166	7.5	15/15	2/15	8/15	Moderate–severe
13. High thiamin	166	170	8.0	15/15	4/15	10/15	Moderate
14. No pyridoxin	167	121	6.2	14/15	0/14	1/14	None
15. No pyridoxin	171	135 †	6.7 †				
High pyridoxin		169	7.8	14/14	1/14	7/14	Mild–moderate
16. 18 per cent casein	178	187	8.5	15/15	2/15	8/15	Moderate–severe
17. 18 per cent casein High pyridoxin	178	196	8.0	12/15	2/12	7/12	Moderate–severe

Groups 1 to 11 constitute one series of experiments and are directly comparable; groups 12 to 17 comprise a second series of experiments.

* Survival = Number living over number at start.
Tumors = Number with tumors over number surviving at 4 months.

† After 3 months' feeding of the azo dye.

The effect of pyridoxin.—When the amount of pyridoxin in the control ration was lowered, tumor formation was greatly reduced. The survival of the animals was 100 per cent in the presence of the azo dye when the level of pyridoxin was reduced to 0.2 mgm. per kg. of ration (diet 4, Tables III and IV). The average daily food consumption was high, and hepatic cirrhosis was either absent or very mild. Only one of the 15 animals on the low pyridoxin ration developed a tumor. This apparent cocarcinogenic effect of pyridoxin was confirmed in two other experiments. The same control ration and the same level of the B vitamins were fed as before, and 8 of 15 animals in the control group developed tumors (diet

12, Table IV). When pyridoxin was omitted entirely from the ration, the animals lost weight in the presence of *p*-dimethylaminoazobenzene, but cirrhosis was largely prevented, and only one of 14 animals developed a tumor by the end of 6 months (diet 14, Table IV). However, when pyridoxin was omitted from the ration for only 3 months but was added at a level of 10 mgm. per kg. of ration during the 4th month of feeding the dye and for 2 months thereafter, 7 of 14 animals developed tumors by 6 months, and the degree of cirrhosis was also greater than when pyridoxin was omitted throughout the experiment (diet 15, Table IV).

In a third series of experiments adult rats with large stores of vitamins (see below) were employed. The control ration and the levels of vitamins fed with the azo dye were the same as in diet C of the previous series, and the carcinogen was administered for 6 months instead of the usual 4. Tumors failed to develop in the control group, but 3 of 10 animals that received 10 mgm. of pyridoxin per kg. of ration developed tumors by the end of the 6 month period (Table V).

TABLE V: PRODUCTION OF LIVER TUMORS WITH SYNTHETIC DIETS FED TO RATS THAT HAD BEEN RAISED TO MATURITY ON FORTIFIED MILK

Group	Average starting weight, gm.	Weight at 4 months, gm.	Average food consumption, gm. per rat per day	Survival * at 6 months	Tumors * † at 6 months	Cirrhosis at 6 months
1. Control, diet C	227	247	11.0	10/15	0/10	None
2. Control, no nicotinic acid, no choline	229	240	10.5	7/15	0/7	Very mild
3. Low riboflavin	249	233	9.5	11/15	8/11 ‡	Mild
4. High riboflavin	216	230	12.0	8/15	0/8	None
5. Low pyridoxin	227	214	9.7	10/15	0/10	None
6. High pyridoxin	242	222	10.0	10/15	3/10	Very mild
7. Low pantothenate	231	223	10.3	7/15	0/7	Mild

* Survival = Number living over number at start.
Tumors = Number with tumors over number surviving at 4 months.
† Tumor incidence in this series was unchanged after an additional 2 months (total = 8 months) in the absence of the dye.
‡ The only tumor appearing in the entire series at 4 months was in this group.

Previous nutritional history.—The animals employed in most of our studies had been raised on crude diets relatively low in vitamins, and they were obtained directly from the Sprague-Dawley colony. Such rats readily developed tumors when they were subsequently fed the synthetic diet (diet C) containing *p*-dimethylaminoazobenzene. However, this diet failed to produce tumors when fed for 6 months to rats that had been raised to maturity on reconstituted milk fortified with crystalline B vitamins. The various modifications of the carcinogenic synthetic diet are indicated in Table V. Tumors appeared in only 2 of the 7 groups, those fed high amounts of pyridoxin and those fed low amounts of riboflavin (diets 3 and 6, Table V).

DISCUSSION

The means by which dietary constituents alter the carcinogenic potency of the azo dyes is as yet unknown, but at least three possibilities suggest themselves. For one thing, the dietary factor may either hasten or retard the destruction of the carcinogen. György and his associates (7) observed that diets made highly rancid with crude linoleic acid appeared to be anticarcinogenic, but that the dye was actually destroyed in the ration *in vitro*. However, no such destruction was observed in any of the diets employed in our laboratories. All uniformly contained 5 per cent of corn oil, which is rich in antioxidants (15). The rations were mixed at frequent intervals and stored in the cold. Dietary constituents might alter the stability of the dye in the digestive tract, and they might also affect its rate of absorption. However, liver is reported to prevent the formation of tumors due to the intraperitoneal administration of the carcinogen (17).

The destruction of the carcinogen within the body is a more likely possibility. Should this destruction prove to be oxidative, the greatest protection against the dye should result from dietary factors directly involved in physiological oxidations. Riboflavin and nicotinic acid seem to fall within this category, although thiamin, which is indispensable in the oxidative catabolism of carbohydrate, did not alter the carcinogenicity of *p*-dimethylaminoazobenzene.

Another destructive pathway is suggested by the observation that the methyl groups of *p*-dimethylaminoazobenzene may function to prevent hemorrhagic kidneys in choline-deficient rats (9); a dietary factor capable of serving as a methyl acceptor might retard tumor formation by demethylating the carcinogen. Nicotinic acid, which is largely excreted as the methylated derivative trigonelline (8), retards tumor

formation somewhat (11), and in our present experiments the absence of nicotinic acid from the diet appeared to increase tumor incidence.

Another consideration involves the high degree of specificity that the azo dyes exhibit by forming only liver tumors, a specificity all the more remarkable since the liver is so highly resistant to the action of the carcinogenic hydrocarbons (22). The liver is the site of many normal metabolic reactions of importance to the organism as a whole, and hence possesses specialized enzymatic mechanisms. The liver is also the site of many detoxification reactions. Hence it is possible that in the process of being itself detoxified, the dye alters an essential liver constituent and that the protective dietary factor tends to maintain that constituent at an adequate concentration, even in the face of the detoxification reaction. Riboflavin may have some such function, since excretory losses of this vitamin are increased when the azo dye is fed (11). Perhaps the carcinogen reacts with one of the proteins to which riboflavin is normally bound in the liver.

A third possibility is that the dietary factor enters directly into the carcinogenic process itself. Presumably the essential carcinogenic reaction is a conversion of ordinary tissue protein into an altered but reproducible type (14). The split products of the carcinogenic azo dyes inhibit the activity *in vitro* of several enzyme systems (10, 12, 20) including the very simple one urease-urea. It has been suggested that this inhibition is due to a combination of the oxidized split product with the sulfhydryl group of the enzymatically active protein (20). The role of the B vitamins in such a reaction is at present obscure and would appear to be at best of only secondary importance. In fact, no enzymatic activity whatever has as yet been demonstrated for pyridoxin. Incidentally, results on the cocarcinogenic effect of pyridoxin on liver tumors due to *p*-dimethylaminoazobenzene should not be extended to other tumor types until the appropriate experiments have been performed.

It is curious that most of the diets employed for the consistent production of tumors with *p*-dimethylaminoazobenzene are nutritionally abnormal. Thus both White and Edwards (25, 26) and György, Poling, and Goldblatt (6) employed diets very low in protein and high in fat. The Japanese were most successful with multiple-deficient diets, and their rice-carrot mixture (13, 18, 19) or imitations thereof (11, 16, 23) have frequently been employed in this country. Thus the over-all effect of diet on hepatic tumor production might seem to be that factors which tend to correct the initial deficiency of the diet also tend to reduce the carcinogenicity of the azo dyes. In our present experiments, however, tumors were most readily produced when the levels of all the B vitamins were high, and the addition of factors like thiamin hydrochloride, calcium pantothenate, or choline chloride to diets low in these factors offered no anticarcinogenic effect whatever.

In general, diets that failed to protect an animal against severe nodular cirrhosis also failed to protect against hepatic tumor formation. However, on several diets (5, 9, 10, 15, Table IV) cirrhosis was relatively mild even though the incidence of tumors was high.

SUMMARY

1. Thirty-six groups of 15 rats each were fed highly purified diets containing *p*-dimethylaminoazobenzene and crystalline synthetic B vitamins. The dye was fed for 4 months and the livers were inspected by laparotomy at 4 and 6 months. The incidence of tumors was low when moderate amounts of the vitamins were fed. These amounts, however, were adequate for the maintenance of adult rats for 6 months. When the levels of all the B vitamins were raised well above the amounts necessary for maintenance, the tumor incidence in the presence of the azo dye reached 66 per cent at 6 months.

2. Under the conditions of the experiment the incidence of tumors due to *p*-dimethylaminoazobenzene was greatly lowered when the level of pyridoxin in the diet was reduced, or when the vitamin was omitted entirely. Large amounts of pyridoxin fed to resistant rats tended to increase the incidence of tumors.

3. The addition of large amounts of riboflavin completely prevented the appearance of tumors in rats receiving only 12 per cent of casein. At carcinogenic levels of the synthetic B vitamins, the incidence of tumors was essentially the same with 12 per cent as with 18 per cent of casein in the diet.

4. The production of tumors appeared to be more difficult in animals raised to maturity on a diet consisting of fortified milk than in animals raised on a diet relatively low in the B vitamins.

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Biocatalysts in Cancer Tissue

II. Inhibition of the Succinoxidase System by Tumor Extracts*

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That certain tumor tissues are low in succinic dehydrogenase activity was first reported by Elliott and Greig (3). Elliott (2) later pointed out that the low values may be due to (a) the possibility that the concentration of this enzyme is really low in tumor, or (b) the possibility that a normal concentration of enzyme exists but that it has been partly inactivated by an inhibitor found in certain normal and tumor tissues.

According to Elliott (2) extracts from tumor tissues (Walker carcinosarcoma 256, Philadelphia No. 1 sarcoma, and Jensen sarcoma) and from certain normal tissues (especially pancreas and spleen) when added to liver, kidney, brain, and muscle suspensions inhibit the activity of the succinoxidase system, the greatest measurable effect being exerted upon the succinic dehydrogenase. Elliott reported, furthermore, that the inhibitor acts on the succinoxidase system in a progressive manner, the inhibition increasing with time; and the inhibition is more pronounced from the start if the liver suspension is treated with the tumor extracts before the succinate is added. In addition, according to Elliott, the inhibitor is nondialyzable and to some extent thermostable. The inhibitory action of the pancreas extracts could be imitated with dilute solutions of commercial trypsin. On the basis of all these findings, Elliott concluded that the inhibitor is probably a proteolytic enzyme and may account in part for the low values observed for succinoxidase activity in certain tumor tissues.

If such an inhibitor for an important, normally occurring enzyme system really exists in tumor tissue, additional information concerning its nature is of prime importance. The present investigations were undertaken to learn more about this inhibitor, the conditions under which it occurs, whether it is found in other tumors, whether the inhibitor from tumor is similar to that from pancreas, and, finally, whether valid assays for the succinoxidase system can be made on tumor tissue.

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EXPERIMENTAL

Enzyme system.—The test system used in the experiments to be described was a succinoxidase preparation from liver. Such preparations approximate the high activity of the enzyme system *in vivo* (8, 9). Rat liver homogenates were freshly prepared and kept in ice water until pipetted. The reaction mixtures contained 0.8 ml. of 0.1 *M* sodium phosphate pH 7.4, 0.4 ml. of 10^{-4} *M* cytochrome *c*, 0.3 ml. of 4×10^{-3} *M* $CaCl_2$, 0.3 ml. of 4×10^{-3} *M* $AlCl_3$, 0.3 ml. of 0.5 *M* recrystallized sodium succinate pH 7.4, 0.1 ml. of either a 5 or 10 per cent liver homogenate in either 0.033 *M* sodium phosphate pH 7.4 or distilled water, plus water and pancreas, tumor, or treated liver homogenate to give a final volume of 3.0 ml. About once a week the calcium, aluminum, and cytochrome solutions were made up into one solution of which 1 ml. was pipetted into each flask. During the course of the investigations, crystalline ribonuclease, trypsin, and chymotrypsin were used (secured through the courtesy of Dr. Kunitz, of The Rockefeller Institute) as well as crystalline insulin. The trypsin and chymotrypsin preparations contained about 50 per cent $MgSO_4$ and were controlled appropriately. For use in experiments, the trypsin and chymotrypsin were made up in 0.001 *N* H_2SO_4. The rate of oxygen uptake was measured in a conventional Warburg apparatus at 38° C. The enzyme system as set up will not oxidize succinate beyond the fumarate stage. Except when otherwise indicated, the inhibitors were incubated with the liver homogenate for one hour prior to testing.

Inhibition by pancreas and pancreatic enzymes.—The data shown in Table I confirm Elliott's (2) observation that extracts from pancreas inhibit the succinoxidase system in liver; they show also, as Elliott pointed out, that the inhibition with pancreas is progressive and that the inhibition is greater if the addition of succinate is delayed. With the delayed addition of succinate, almost complete inhibition of succinoxidase activity was obtained with a ratio of 3 parts of pancreas to 10 parts of liver. The results with crystalline trypsin shown in Table I also confirm Elliott's observations with commercial trypsin.

Chymotrypsin also inhibits to about the same extent as trypsin.

That all the inhibitory activity of the pancreas cannot be due to trypsin or chymotrypsin is also shown by the data in Table I, for even with 10 mgm. of either pure trypsin or chymotrypsin one does not attain as great an inhibition as that obtained with only 3 mgm. of moist pancreas. Some of the inhibition may be due to ribonuclease, which inhibits succinoxidase activity, as can be seen from Table I. It is also of interest to note that crystalline insulin appears to inhibit succinoxidase activity. This inhibition, however, may not be caused by insulin *per se* but by

TABLE I: INHIBITION OF LIVER SUCCINOXIDASE BY PANCREAS AND PANCREATIC CONSTITUENTS

Test system as described in text (10 mgm. liver homogenate)

Inhibition calculated from oxygen consumed per 10 minute interval

Experiment No.	Inhibitor	Inhibitor concentration, mgm. per flask	Inhibition, per cent			
			Substrate added at beginning		Substrate added at 20 min.	
			10-20 min.	30-40 min.	10-20 min.	30-40 min.
809	Pancreas	1.0	6	13	78	89
		2.0	49	78	84	97
		3.0	74	92	95	95
		4.0	87	93	96	100
		5.0	90	95	95	95
810	Trypsin	0.1	−19	−18		
		1.0	13	22		
		5.0	40	58		
		10.0	56	72		
	Chymotrypsin	0.1	−4	−7		
		1.0	5	14		
		5.0	38	45		
		10.0	58	69		
1126	Ribonuclease	0.3			33	31 *
		0.9			58	58
1125	Insulin †	1.5			68	55 *
		3.0			78	70
		5.4			89	85

* Incubated for 1 hour with liver homogenate prior to tests.

† Test system contained 5 mgm. of liver.

minutes by control, 41.6, 42.0 μl.; by 30 mgm. of tumor, 10.3, 10.7 μl.; by liver plus tumor, 52.0, 51.8, 51.5, 52.5 μl. It is obvious that the total oxygen consumed is the sum of the individual quantities taken up by liver and tumor and that the tumor has exerted no inhibitory effect on the liver succinoxidase system. When the ratio of tumor to liver was increased to 10:1, an inhibition of liver succinoxidase to the extent of only 10 per cent occurred.

Since no inhibitory effects were obtained with healthy tumor tissue, it seemed possible that Elliott's results might have been obtained by the inclusion of some necrotic tissue, which conceivably might inhibit liver succinoxidase. Accordingly, experiments were carried out with younger and older tumors (the younger ones presumably having less necrotic tissue than the older ones), and the healthy tissue from the outside was carefully separated from the inner necrotic tissue. In every case, homogenate equivalent to 60 mgm. of tumor was added to 5 mgm. of liver for 1 hour at 37° C. prior to testing in the Warburg vessels. The results of such a series of experiments are shown in Table II. A number of important conclusions may be drawn from these data. First, the healthy tissue from the outside of the tumor gives little or no inhibition. Second, the only tissue which gives inhibition to any appreciable extent is the necrotic tissue from the inside of the tumor, this inhibition in-

the presence of zinc in the crystalline preparation since succinic dehydrogenase is inhibited in the presence of zinc (10).

Inhibition by tumor homogenates.—Whereas the results reported in this paper with pancreas and trypsin completely confirm those of Elliott, the first results obtained with tumor tissue (Walker carcinosarcoma 256, 7 to 10 days old) were completely negative, whether the tissue was incubated with the liver homogenate before addition of the succinate or not. In one typical experiment, with Walker 256 about 7 days old, when the necrotic tissue was carefully scraped away before homogenization and 30 mgm. of tumor were added to 10 mgm. of liver the following results were obtained: average O_2 consumed per 10

creasing with the age and, consequently, the necrosis of the tumor.

It appears, therefore, that Elliott's reported inhibition of liver succinoxidase may have been due to the necrotic tissue rather than to the healthy tissue. Incidentally, it should be pointed out that the necrotic tumor tissue in all cases has a lower succinoxidase activity than does the adjacent healthy tumor tissue. This fact may explain the wide variation in the succinate Q_{O_2} values in the tumors studied by Elliott and Greig. Also, it is important to point out that any given tumor of sufficient age may or may not give inhibition depending upon what portion is tested.

TABLE II: INHIBITION OF LIVER SUCCINOXIDASE BY VARIOUS TUMOR HOMOGENATES TREATED AS INDICATED

Test system as described in text (5 mgm. liver homogenate)

Inhibitor concentration per flask 60 mgm.

Experiment No.	Tumor	Average inhibition, per cent: Outer tissue	Average inhibition, per cent: Inner tissue
1011	Walker 256 (13 days old; very little necrosis)	6	17
1017	Walker 256 (17 days old; much necrosis)	1	43
	Heated 100° for 10 minutes	28	31
	Dialyzed in distilled water at 4° C. for 24 hours	38	61
	Heated, dialyzed	43	60
	Autolyzed, 37° C. for 24 hours	68	77
1006	Flexner-Jobling (14 days old; no necrosis; inner tissue cheesecake in consistency)	1	7
1026	Flexner-Jobling (18 days old; some necrosis; beginning liquefaction)	0	17
	Heated 100° for 10 minutes	11	8
	Autolyzed at 37° for 24 hours	42	20
	Dialyzed in distilled water 24 hours	18	
	Set in ice chest 24 hours	21	
	Heated, dialyzed	10	
1124	Jensen sarcoma (10 days old; little necrosis; no liquefaction)	0	18
	Heated 100° for 10 minutes	7	13
1007	Jensen sarcoma (14 days old; much necrosis; considerable liquefaction)	8	52
	Heated 100° for 15 minutes	14	24

When tissue is deprived of an adequate blood supply, as is that in the interior of a tumor, it undergoes necrosis. The reason is not completely clear, but it appears that one of the factors responsible for the tissue breakdown is the release of autolytic enzymes of the cathepsin type. It is conceivable that these enzymes, which would be particularly abundant in a well necrosed tissue, might be the agents mainly responsible for the inhibition of the liver succinoxidase. If such an interpretation is correct, it should be possible to take healthy tumor tissue that does not act as an inhibitor and allow it to undergo autolysis, after which it should inhibit. That such an interpretation is correct is indicated by the experiments (1017 and 1026) with autolyzed tumor included in Table II. Tumor tissue that originally gives no inhibition on autolysis does inhibit the succinoxidase system.

Inhibition by liver.—If the inhibition of liver succinoxidase is due in part to the liberation of catheptic enzymes as indicated above, then autolyzed tissue from other sources, including liver itself, should inhibit. That this is true is shown by the data in Table III in which liver succinoxidase is almost completely inhibited by autolyzed liver.

These observations make the results with pancreas, trypsin, and chymotrypsin fit into the same kind of pattern as those with tumor, since in both cases inactivation of the succinoxidase system appears to be associated with proteolytic enzymes.

TABLE III: INHIB.TION OF LIVER SUCCINOXIDASE BY LIVER HOMOGENATE TREATED IN VARIOUS WAYS

Test system as described in text (5 mgm. liver homogenate per flask)

Inhibitor concentration per flask 50 mgm.

Experiment No.	Treatment	Average inhibition, per cent
1021	Untreated	0
	Autolyzed 24 hours, 37° C.	98
	Heated 100° for 10 minutes	26
	Dialyzed 24 hours in distilled water	14
	Dialyzed 24 hours in buffer, pH 7.4	11

Mechanism of inhibition.—If both the inhibitors from pancreas and the succinic dehydrogenase have the SH structure, it is possible that at least part of the inhibition by pancreas may be caused by the formation of the following type of complex: enzyme · S · SR, where R represents any SH compound present in pancreas since this appears to be the mechanism by which cysteine and glutathione inhibit succinic dehydrogenase (10). Such complex formation would prevent succinate from approaching the enzyme surface and thus produce inhibition. If such an interpretation is correct, it should be possible to inactivate the inhibitors of pancreas, at least in part, by first incubating pancreas with an SH poison, and then adding it to liver homogenate. That this can be done is shown by the results in Fig. 1, in which the Q_{O_2} for successive 10 minute intervals is plotted against time. By this method of plotting, the data in effect show the amount of active succinic dehydrogenase at any given time (10). It will be observed that there is a slow spontaneous decrease in activity in the control, which consisted of the liver succinoxidase test system alone.

When either quinone or pancreas was also present in the test mixture, the rate of inactivation was much greater and was nearly complete after 60 minutes. However, when both were present the inhibition was much less than with either inhibitor alone, indicating reaction between the quinone and pancreas to yield a less toxic preparation. The data in Fig. 1 are typical of those in the tables and show the magnitude of the absolute quantities from which the percentage inhibitions were calculated and reported in the tables.

From the results presented above, it appears that at least part of the inhibition produced by the pancreas may be associated with the formation of —S—S—

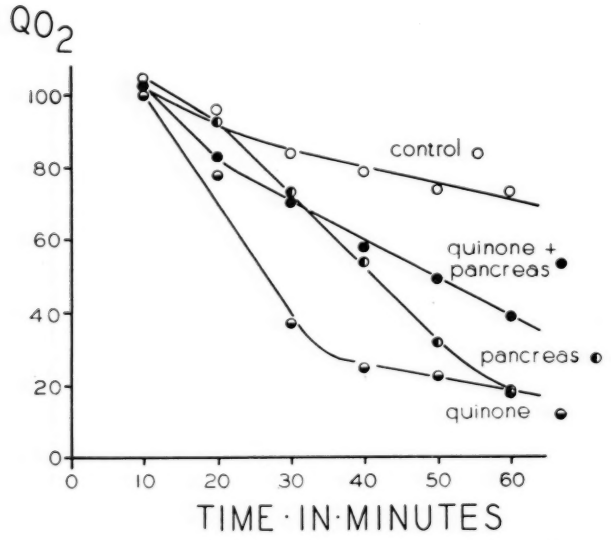

FIG. 1.—Relationship between Q_{O_2} (cu. mm. O_2 uptake per mgm. dry liver per hour) and time for liver succinoxidase alone and when treated with quinone, pancreas, and quinone-treated pancreas. The quinone-pancreas was prepared by incubating the quinone with the pancreas for 1 hour at 37° C. prior to testing.

Concentration of pancreas, 2 mgm.; quinone-final, $M/30{,}000$ (10.8γ); liver, 5 mgm. The control consists of the succinoxidase test system as described in the text, with 0.1 ml. of a 5 per cent liver homogenate as the source of the succinoxidase.

linkages between succinic dehydrogenase and SH compounds occurring in pancreas. Grassmann, Dyckerhoff, and von Schoenebeck (4) report that pancreatic trypsin is inhibited by cysteine. This suggests that trypsin may be an SH enzyme and that the mechanism of inactivation with cysteine may involve the formation of a complex of the type mentioned above: trypsin—S—S—Cy. It is possible that the inactivation of a portion of the inhibitors from pancreas may be due to the inactivation of trypsin, which can no longer function as an inhibitor.

That the inhibition by pancreas involves more than a simple interaction between SH groups, however, is indicated by the fact that heated pancreas produces less inhibition than untreated pancreas (Table IV). Indeed, If Mirsky (7) is correct in pointing out that the denaturation of a protein (such as would occur on heating) unfolds the protein so that SH groups that were not exposed in the native protein become exposed in the denatured protein, one might observe greater inhibition than before, rather than less, if the inhibition were due only to nonspecific sulfhydryl interaction. On the other hand, if part of the inhibition depends upon the integrity of a functional proteolytic enzyme (whether from pancreas or tumor), then one should get less inhibition following heating. The data on heated pancreas show that part of the inhibition of succinoxidase activity by pancreas requires the integrity of pancreatic enzymes that are destroyed by heating. Apparently the initial formation of a succinic dehydrogenase-proteolytic enzyme complex may be dependent upon the presence of SH groups, while the hydrolysis of the enzyme protein depends in addition upon the native proteolytic enzyme. The latter would be expected to be destroyed by heating. This interpretation fits in with the experiments of Bernheim (1), who showed that the succinic dehydrogenase may be inactivated before considerable hydrolysis occurs, suggesting the formation of a complex, followed by proteolysis.

TABLE IV: INHIBITION OF LIVER SUCCINOXIDASE BY PANCREAS HOMOGENATE TREATED IN VARIOUS WAYS

Test system as described in text (10 mgm. liver homogenate)

Experiment No.	Inhibitor	Inhibitor concentration, mgm. per flask	Average inhibition, per cent
828	Pancreas	4	98
	Pancreas, heated 100° for 10 minutes	4	15
829	Pancreas	5	95
	Pancreas, heated 100° for 10 minutes	5	16
	Pancreas, dialyzed	5	95
	Pancreas, heated, dialyzed	5	13

Results similar to those with pancreas are obtained with the "inhibitors" from tumor (Table II, see especially experiments 1017 and 1026). Heating to 100° C. destroys part of the inhibition but not all. It is suggested that the portion remaining is due to the presence of free SH groups on tissue proteins exposed by heat denaturation. That these SH groups are not on low molecular weight compounds of the glutathione type is also indicated by the data in Tables II and IV, where it is shown that they are not dialyzable after heating. The data (Tables II and III) also show that heating confers on tissues unable to inhibit succinic dehydrogenase in the living state inhibitory powers exactly like those possessed by necrotic tissue after heating.

Dialysis apparently also produces denaturation of protein and thus exposes SH groups that may inactivate the succinic dehydrogenase. In the case of tumor (see Table II, experiments 1017 and 1026) dialysis causes greater inhibition than heating for 10 minutes at 100°. This increase in the inhibitor activity cannot depend upon the liberation of autolytic enzymes since heating to 100° for 10 minutes does not destroy this additional inhibitor. This type of phenomenon does not seem to occur in liver (Table III). That the inhibition produced by heated liver is probably due to SH groups exposed on protein molecules resulting from denaturation is indicated by the work of Stotz and his associates (11), who showed that heating extracts from guinea pig liver increased the number of nondialyzable SH groups.

DISCUSSION

The results presented above indicate that, with respect to tumor tissues, Elliott's inhibitor [1] is found only in necrotic tumors or in tumor tissue that has been allowed to undergo autolysis. This fact emphasizes the importance of determining succinic dehydrogenase in healthy tumor tissue and suggests that such tests would be valid.[2]

The nature of the inhibitor appears to be complex and to include several factors, which occur in different proportions according to the source of the inhibitor and its treatment. Elliott's statement that the inhibitor is probably a proteolytic enzyme is essentially correct as far as whole tumors are concerned, but this inhibition appears to be negligible in tumors free from necrosis. We have been able to work with much smaller tumors than Elliott used because the sensitivity of the succinoxidase test system has been greatly increased (8, 9) since Elliott's experiments were completed. In necrotic or autolyzed tumors, or in autolyzed liver, the inhibitor activity is probably associated with proteolytic enzymes. The activity remaining when these sources are heated probably depends upon the liberation of fixed SH groups. In pancreas, the inhibitor appears to be due to both proteolytic enzymes and to ribonuclease.[3] In heated pancreas, the inhibitor activity is probably caused by both ribonuclease and fixed SH groups, since ribonuclease is remarkably heat-stable (5). The inhibition observed by Elliott in certain normal tissues may be due in part to ribonuclease, since the only normal tissues that contained appreciable amounts of inhibitor were pancreas and spleen; and these tissues contain much more ribonuclease than other normal tissues, according to unpublished studies by Mr. J. A. Bain and Dr. H. P. Rusch of this Institute.

[1] Elliott reported that heart muscle preparations were not inhibited by the tumor extracts but were, in fact, considerably stimulated; and he suggested that the succinoxidase system in heart muscle differed from that in other tissues. The recent work of Swingle, Axelrod, and Elvehjem (12, Table III) demonstrates that heart muscle is unable to destroy its coenzyme I even in the presence of added calcium ions at a rate sufficient to prevent toxicity to its succinoxidase, when succinate is added at the beginning of the experiment. This is in contrast to liver and brain, which possess sufficient nucleotidase to prevent the inhibition of the succinoxidase. The stimulation observed by Elliott in the case of heart succinoxidase plus tumor extracts is adequately explained by the presence of nucleotidase in the latter, and there is now no necessity to assume that the heart succinoxidase is any different from that in other tissues.

[2] The results of such a survey will be reported at an early date.

SUMMARY

Experiments have been carried out on an inhibitor from tumor tissues with a succinoxidase preparation from liver as a test system. The results of these experiments may be summarized as follows:—

1. Healthy tumor tissues contain no inhibitor.

2. Necrotic tumor or healthy tumor after autolysis possesses such an inhibitor.

3. Liver, after autolysis, also shows inhibitor activity.

4. Pancreas, as well as crystalline trypsin, chymotrypsin, and ribonuclease, inhibits succinoxidase activity.

5. The inhibitory action is not completely destroyed by heating, and it is suggested that the heat-stable inhibition may be due to SH compounds and to ribonuclease in certain tissues.

6. It is concluded that succinoxidase assays on healthy tumor tissues would not involve inhibitor action and would therefore be completely valid.

We wish to acknowledge the helpful criticism of Dr. K. A. C. Elliott who read this manuscript before its final revision.—AUTHORS.

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[3] Although ribonuclease is believed to act specifically in hydrolyzing ribonucleic acid (5), Loring (6) has shown that it inactivates the tobacco mosaic virus, which is a ribonucleoprotein. It is thus possible that the succinoxidase system may include a ribonucleoprotein. Experimental data bearing on this problem will appear in a separate publication.

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Experimental Fibroids in Hypophysectomized Female Guinea Pigs*

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In the problem of the experimental production of fibroids in the guinea pig with estrogen (12, 3, 11) it was of interest to investigate the possible participation of the pituitary gland. Does the tumor-inducing effect of estrogen act through the hypophysis? Is it possible to induce experimentally fibroid tumors in the hypophysectomized guinea pig or is the presence of the anterior hypophysis indispensable? If fibroids occur in the hypophysectomized animal, will the fibroid reaction be the same as in the nonhypophysectomized animal?

EXPERIMENTAL PROCEDURE

Material and technic.—A group of 21 hypophysectomized female guinea pigs was treated with estrogen and compared with another group of 15 estrogen-treated nonhypophysectomized female guinea pigs. Tables I and II summarize the material used. The guinea pigs were adults weighing between 380 and 640 gm., and were derived from inbred strains. Because ovariectomy enhanced the fibroid tumor reaction (5, 6) all except 3 animals of both groups were ovariectomized. The estrogen was administered as subcutaneous pellets of α-estradiol.[1] The pellets, which weighed between 5 and 10 mgm., were made according to the technic of Forbes (1).[2] They were implanted on the day of castration, usually from 4 to 51 days after hypophysectomy. In some cases hypophysectomy, ovariectomy, and estradiol implantation were done at the same time.

Hypophysectomy was performed according to the parapharyngeal technic of McPhail and Parkes (10), under ether anesthesia. Like these authors, we noted a very high death rate, especially from the 2nd to the 4th day after the operation. After trying different ways of insuring survival, we found that omission of the tracheal cannula prevented pulmonary sequelae and increase in the amount of water drunk decreased the death rate. After operation the animals were housed in a special room kept at 25° C. They were fed in the same manner as the nonhypophysectomized ones, with fresh vegetables and prepared food pellets (B-B-Laboratory rabbit diet A).

All the animals were periodically weighed, and the estrogen-induced reactions of the nipples and vagina were observed. At autopsy the uterus and one mammary gland were removed for histological examination. The sella turcica was carefully dissected. Bouin fixation was used.

Control of the hypophysectomy.—The completeness of the hypophysectomy was determined by microscopic examination of serial sections of the sella turcica. Standard staining was employed and horizontal and coronal sections were made. The pars tuberalis, pars intermedia, and pars anterior were especially studied. The study of the sella turcica was correlated with the study of the mammary gland, since Lyons and Pencharz (9) and Gomez and Turner (2) have demonstrated that in the completely hypophysectomized guinea pig treated with estrogen, the mamma does not develop.

Estimation of the result.—All the fibroids in each animal were recorded by sketches drawn to actual size, and the three types of estrogen-induced reaction, namely fibrosis, disseminated miliary nodules ("seed tumors"), and fibroid tumors, were rated according to the system of Lipschütz and Vargas (7). This gave a general evaluation of the size, extent, and distribution of the fibroids.

RESULTS

Results of hypophysectomy.—In order to understand easily certain details of the results of hypophysectomy it is useful to recall some peculiarities of the relationship between the pars anterior and the pars intermedia in the guinea pig. The so called pars anterior in the guinea pig is actually the pars inferior. The pars intermedia has a special prolonga-

* This work is a continuation of research done with Dr. A. Lipschütz in Chile.

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[1] The estradiol was generously supplied to the Director, Dr. G. W. Corner, by the Ciba Pharmaceutical Products, Inc., through the courtesy of Dr. E. Oppenheimer.

[2] We wish to express thanks to Dr. T. R. Forbes for the facilities given for making the pellets.

tion caudad, which partially covers the pars posterior (Vanderburgh, 13) and is very difficult to remove completely. Fig. 1 illustrates these anatomical relations.

Table III gives the results of hypophysectomy in 14 animals. In 8 cases no pars anterior tissue was found. In 6 of these the pituitary stalk and the pars eosinophilic cells and arranged as solid cords separated by sinusoids. These fragments lay just outside the lateral regions of the stalk, and were included in a highly vascular connective tissue in very close relation with the periosteum. It is almost impossible to remove such pieces at operation.

TABLE I: RELATIVE FIBROID RESPONSE AND MAMMARY RESPONSE IN HYPOPHYSECTOMIZED ADULT FEMALE GUINEA PIGS BEARING SUBCUTANEOUSLY IMPLANTED PELLETS OF ESTRADIOL

Animal No.	Body weight		Period hypophysectomized, days	Period treated, days	Amount absorbed weekly, mgm.	Genital effects			Fibroid response, 0–12 *	Mammary response		Remarks
	Start, gm.	End, gm.				Continuous vaginal opening	Genital bleeding	Uterine weight, gm.		Growth of duct acini, 0–12 †	Growth of nipples, mm.	
HYPOPHYSECTOMIZED AND OVARIECTOMIZED												
(a) Completely Hypophysectomized												
4	460	400	34	14	0.70	+	0	1.8	0	0	0.5	Dying. Killed
1	400	340	19	17	0.29	+	+	1.9	1.0	0	1.0	Killed
8	440	390	8	19	0.40	+	+	3.5	1.5	0.5	0	Found dead
23	560	500	51	20	0.10	+	0	3.4 ‡	5.0	0	3.0	Dying. Killed
10	380	380	6	25	0.34	+	+	3.6	3.0	0	1.0	Killed
2 §	425	430	17	36	0.23	+	0	3.5	2.0	0	6.0	Killed
7	450	475	15	59	0.28	+	+	—	10.0	0	1.0	Killed
9	430	450	8	69	0.29	+	+	7.0	7.0	0	6.0	Killed
Average . .	445	420	—	32	0.33	—	—	3.4	3.7	0	2.3	
(b) Incompletely Hypophysectomized												
20	500	360	0	10	0.42	+	+	2.1	1.0	4.0	2.0	Found dead
21 §	320	320	0	20	0.17	+	+	—	0	—	3.0	Found dead
6 §	470	360	9	26	0.42	+	+	1.6	1.0	—	1.0	Found dead
19 §	535	450	0	27	0.18	+	0	2.7	2.0	8.0	6.0	Dying. Killed
15	425	320	4	32	0.26	+	0	3.1	3.0	4.0	0.5	Killed
22	625	460	26	47	0.27	+	0	2.5 ‡	5.0	2.0	4.0	Found dead
14	420	400	4	49	0.15	+	+	—	5.5	3.0	2.0	Dying. Killed
5 §	510	475	32	51	—	+	0	4.5	9.0	3.0	3.0	Killed
11	480	340	14	54	0.22	+	+	9.1 ‡	9.0	2.0	0.5	Found dead
17	510	480	10	55	0.23	+	+	—	7.5	5.0	5.0	Found dead
13	470	460	4	66	—	+	+	10.1 ‡	5.0	3.0	0.5	Found dead
Average . .	480	395	—	39	0.26	—	—	2.8	4.3	3.4	2.5	
HYPOPHYSECTOMIZED AND NONOVARIECTOMIZED												
Completely Hypophysectomized												
106 §	640	550	0	19	0.29	+	+	—	0.5	0	2.5	Found dead
109	550	450	46	97	0.20	+	0	10.0	3.5	0	1.5	Found dead

* This includes all the different types of experimental fibroids (fibrosis, miliary disseminated nodules, and fibroid tumors) according to Lipschütz and Vargas (7).

† Figures obtained by grading the response of the ducts, the acini, and the secretion respectively, from 0 to 4 points according to the degree of response.

§ Without control of the sella turcica.

‡ With apical tumors. The average weight does not include these cases.

tuberalis remained. In 7 of the 8 cases microscopic fragments of the pars intermedia remained and in 5 these were found caudad in the sella turcica. This is the critical region, which should be aspirated carefully during operation and studied with particular attention by microscopic examination. In the other 2 of the 8 cases we found microscopic fragments with the appearance of intermediate lobe tissue, without

In some cases it was difficult to determine whether or not a fragment of the pars posterior was attached to the stalk. The correlative study of the mammary gland and the sella turcica was in good agreement. The mamma grew only when a portion of the anterior lobe remained behind. When the removal of this was complete no mammary growth was observed (Fig. 2). We believe that this histologic control of

TABLE II: CONTROL FOR MATERIAL IN TABLE I. RELATIVE FIBROID RESPONSE IN NONHYPOPHYSECTOMIZED ADULT FEMALE GUINEA PIGS BEARING SUBCUTANEOUSLY IMPLANTED PELLETS OF ESTRADIOL

Animal No.	Body weight		Period treated, days	Amount absorbed weekly, mgm.	Genital effects			Fibroid response, 0–12 *	Mammary response		Remarks
	Start, gm.	End, gm.			Continuous vaginal opening	Uterine bleeding	Uterine weight, gm.		Growth of duct acini, 0–12 †	Growth of nipples, mm.	
TREATED OVARIECTOMIZED											
57	450	450	14	0.75	+	0	2.8	0	3.5	3.0	Killed
52	430	480	17	0.74	+	+	2.0	2.0	—	4.5	Killed
59	420	430	20	0.30	+	0	3.2	3.0	4.0	3.5	Killed
68	480	510	20	0.14	+	0	3.0	4.0	4.0	4.0	Killed
60	410	450	25	0.17	+	0	2.8	2.0	—	5.0	Killed
55	400	440	27	0.28	+	0	3.0	4.0	—	6.0	Killed
51	440	480	36	0.23	+	0	3.5	3.0	9.0	4.5	Killed
69	640	670	47	0.33	+	0	7.5 ‡	4.5	—	4.5	Killed
61	480	490	49	0.36	+	0	4.5 ‡	2.0	6.0	6.0	Killed
56	500	420	51	0.23	+	0	8.0	6.5	8.0	6.0	Killed
63	470	455	54	0.27	+	+	5.7	4.5	—	5.5	Killed
54	460	450	59	0.35	+	0	—	6.0	8.0	5.0	Killed
67	565	550	63	0.69	+	0	—	3.5	—	12.0	Killed
58	450	490	69	—	+	0	5.0	4.5	8.5	8.0	Killed
Average . .	470	480	38	0.37	—	—	4.2	3.5	6.5	5.5	
TREATED NONOVARIECTOMIZED											
154	480	500	85	0.19	+	0	8.0	8.0	—	9.0	Killed

*, †, and ‡ have the same meaning as in Table I.

TABLE III: RELATION BETWEEN THE MICROSCOPIC HYPOPHYSIAL FRAGMENTS LEFT DURING HYPOPHYSECTOMY AND THE FIBROID AND MAMMARY RESPONSE ‡

Animal No.	Parts of the hypophysis					Fibroid response 0-12 *	Mammary response	
	Anterior	Intermediate	Posterior	Tuberalis	Stalk		Duct acini growth 0-12 †	Nipple growth, mm.
4	0	+	0	+	+	0	0	0.5
1	0	+	0	0	0	1.0	0	1.0
8	0	+	0	+	+	1.5	0.5	0
23	0	+	0	+	+	5.0	0	3.0
10	0	+	+	+	+	3.0	0	1.0
7	0	+	0	0	0	10.0	0	6.0
9	0	+	±	+	+	7.0	0	1.0
109 §	0	0	0	+	+	3.5	0	6.0
Average						4.0	0	2.5
15	+	+	±	+	+	3.0	4.0	0.5
22	+	+	+	+	+	5.0	2.0	4.0
14	+	+	+	+	+	5.5	3.0	2.0
11	+	+	+	+	+	9.0	2.0	0.5
17	+	+	+	+	+	7.5	5.0	5.0
13	+	+	0	+	+	5.0	3.0	0.5
Average						6.0	3.0	2.0

‡ In no animal was it possible to see any fragment of the hypophysis by binocular microscopic examination. A fragment of the stalk was seen twice.

* and † same as in Table I.

§ Nonovariectomized animal.

the mammary gland in the treated guinea pig is a good test for verifying the completeness of the removal of the anterior lobe. For this reason in 2 cases, V-2 and V-106, where the sella turcica was not examined in serial sections after autopsy, and in which no mammary growth occurred, we considered the hypophysectomy to be complete. In one case no fragment of the anterior lobe was found although the mammary gland showed development, and subsequent microscopic reexamination of the serial sections of the sella turcica demonstrated a minute piece of anterior lobe remaining.

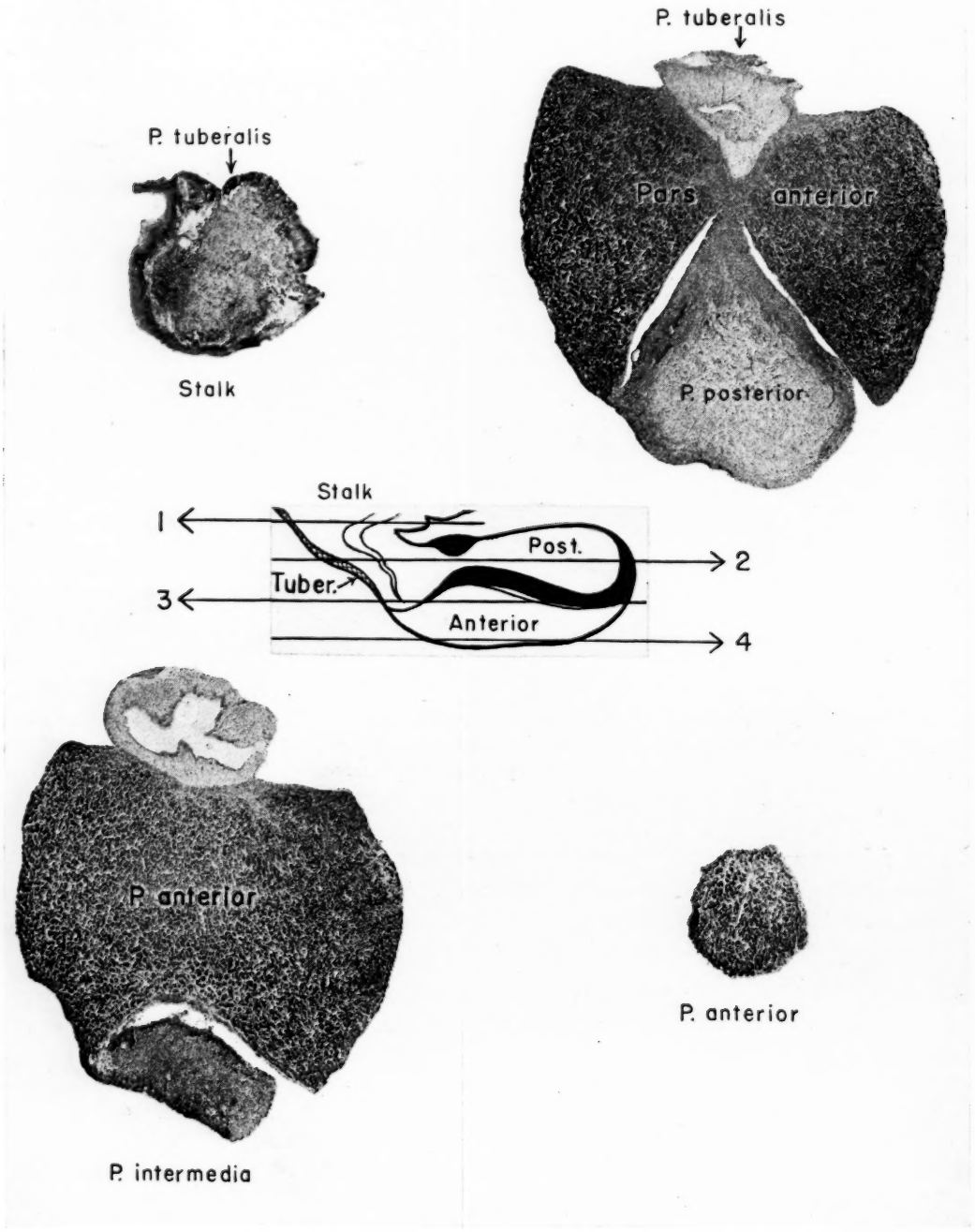

FIG. 1.—Normal hypophysis of the adult female guinea pig. Horizontal sections. The central drawing is taken, with some modifications, from Vanderburgh (13). After hypophysectomy a piece of the intermediate lobe (section 3), situated caudad in the sella turcica can simulate a piece of the anterior lobe. In the cephalad portion of the sella a piece of the stalk, which descends deeply in the guinea pig, can simulate a piece of the posterior lobe. Hematoxylin and eosin. × 12.8.

Fibroid reactions in the completely hypophysectomized guinea pig.—The estradiol was very poorly

tolerated by the hypophysectomized animals. After implantation of the estradiol the body weight decreased and most of the animals died within a few days. This occurred even after complete recovery from operation.

All the control group was killed after periods of treatment comparable to those tolerated by the hypophysectomized animals. After implantation of the estradiol the body weight decreased and most of the animals died within a few days. This occurred even after complete recovery from operation.

All the control group was killed after periods of treatment comparable to those tolerated by the hypophysectomized animals (Tables I to III). In 9 of the 10 animals in which removal of the anterior lobe was complete, fibroids were present (Table I, Fig. 3). The remaining guinea pig, treated for only 14 days, showed no fibroid reaction, as was to have been expected, since this is seldom found before 20 days of treatment.

The qualitative and quantitative study of the fibroids did not show any difference between the hypophysectomized and the nonhypophysectomized animals. Thus, the histological appearance of the fibroids was the same in both groups, confirming the general description of Lipschütz and his coworkers (4, 8). The genital fibroids were of fibromyomatous nature, while the extragenital were almost pure fibromas (Fig. 4). The degree of the reaction was the same for the completely hypophysectomized castrated group (3.7) and for the nonhypophysectomized castrated controls (3.5) The growth, extent, and distribution of the fibroids were similar in both groups of animals (Figs. 5 and 6).

Uterine bleeding occurred more often in the hypophysectomized group than in the controls (Tables I

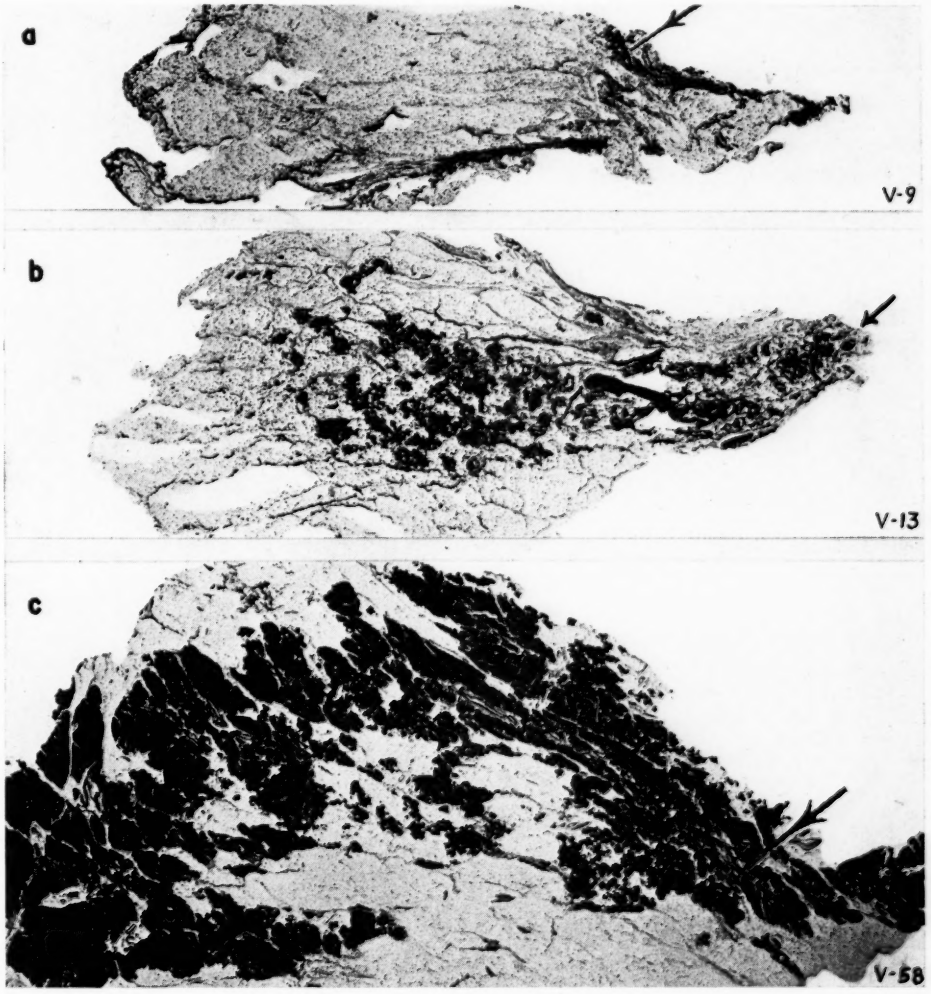

FIG. 2.—Mammary response in the ovariectomized and completely and incompletely hypophysectomized guinea pig, treated with estradiol.

(a) Completely hypophysectomized animal (V-9). Relative mammary response = 0.

(b) Incompletely hypophysectomized animal (V-13). Relative mammary response = 4.

(c) Nonhypophysectomized estrogen-treated control (V-58). Relative mammary response = 8. The arrows indicate the region near the nipple.

The 3 animals were castrated and received pellets of estradiol at about the same time. Hypophysectomy was performed before estradiol was implanted (see Tables I and II). Period of treatment 66 to 69 days. Hematoxylin and eosin. × 5.

and II). Histologic examination of the genital tract of the completely hypophysectomized animals showed alterations similar to those of the control group. The vagina usually had the appearance of the pseudogestational state, without cornification; the cervix was very much enlarged, with pronounced epithelial proliferation. The uterine cornua differed greatly; some were almost normal in the shortest experiment (V-4), others showed metaplasia, cystic proliferation, or total necrosis with pyometra in the more prolonged experiments (V-1, 8, 23, 10, 7, 9, and V-109).

as crucial, demonstrating that these segments take no part in the production of fibroids by estrogen. (Table III). In all the hypophysectomized animals, *i.e.* those in which the pars anterior was completely or incompletely removed, the fibroid reaction occurred in the same manner as in the nonhypophysectomized controls. Moreover, hypophysectomized and nonhypophysectomized estrinized animals behaved in general exactly alike, since both groups were inhibited in growth and in body weight, and since both showed estrogenic and fibroid reactions in similar degree. They differed in one respect only: the mammary gland did not develop in the completely hypophysectomized group. In other words, a contradictory condition occurred in which a high degree of fibroid reaction was accompanied by no mammary growth.

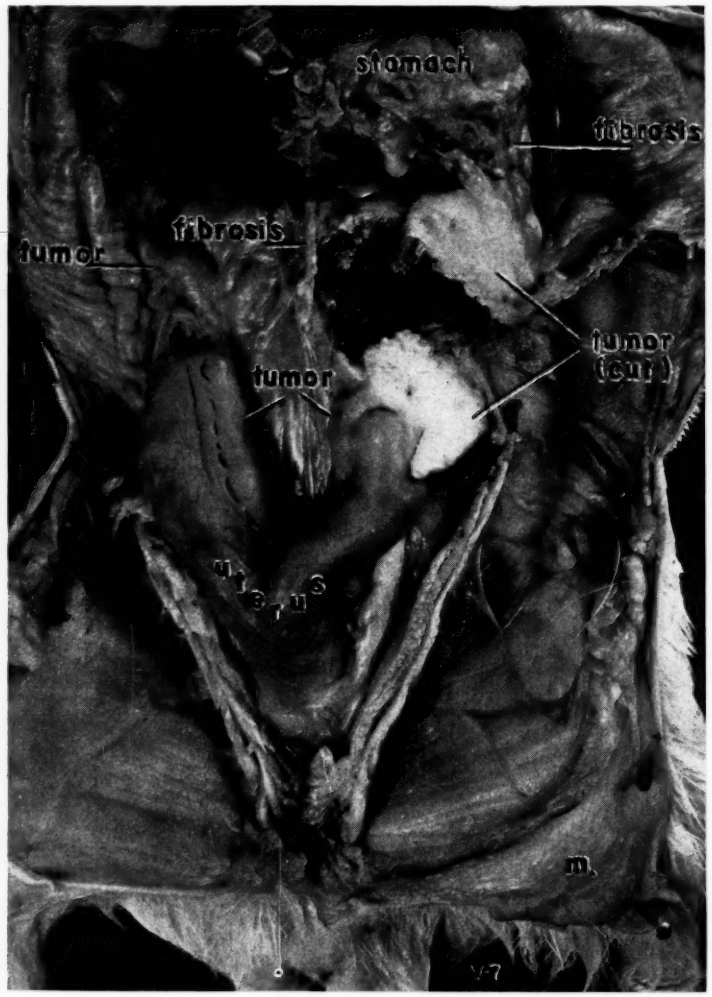

FIG. 3.—Genital and extragenital abdominal fibroids in a completely hypophysectomized ovariectomized guinea pig, treated with a subcutaneously implanted pellet of estradiol (9.8 mgm.). Animal V-7, 59 days of treatment. Fibroid response = 10.0. Mammary response = 0. Natural size.

COMMENT

During hypophysectomy in the guinea pig microscopic pieces of the pars intermedia are frequently overlooked. The pituitary stalk and the pars tuberalis usually remained in the sella turcica, as it is almost impossible to extirpate them completely. Experiments V-1, V-7, and V-109 may be taken as crucial, demonstrating that these segments take no part in the production of fibroids by estrogen.

Thus the fibroid response to estradiol is obviously not exerted through the anterior lobe of the pituitary gland.

SUMMARY

Complete extirpation of the anterior lobe of the hypophysis had no influence on the experimental fibroid reaction in castrated adult female guinea pigs. Neither the intensity, extent, nor distribution of the tumor process was affected by this ablation. Incomplete removal of the anterior lobe gave the same experimental results except for a difference in the development of the mammary gland, since microscopic fragments of the pars anterior permitted mammary growth. Thus the histologic study of the mammary gland was a test for the completeness of the hypophysectomy. Two completely hypophysectomized non-ovariectomized guinea pigs showed the same results with respect to both the fibroid and the mammary response.

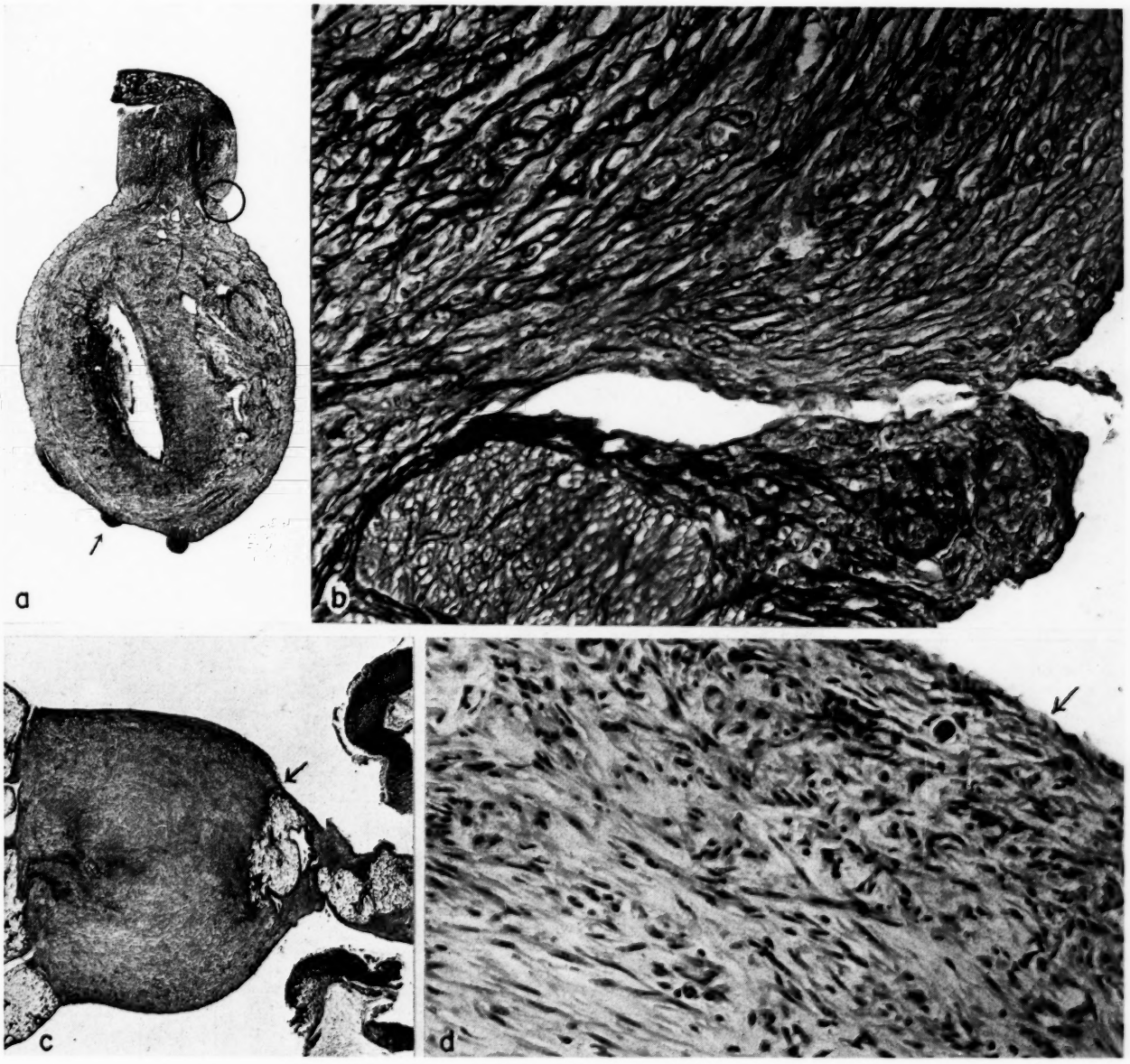

FIG. 4.—Structure of the genital and extragenital abdominal fibroid tumors, in a completely hypophysectomized ovariectomized guinea pig, treated with a subcutaneous pellet of estradiol (= 9.6 mgm.). Animal V-9, 69 days of treatment.

(a) Uterine fibroids at various locations and with different degrees of development. Hematoxylin and eosin. × 5.

(b) Higher power view of the site indicated by a circle in (a), showing fibromyomatous nature of a mesometrial tumor. The smooth muscle cells lie singly among thin fascicles of collagenous fibers. × 200.

(c) Fibroid of the omentum. General view of the tumor; a portion of the stomach is shown without infiltration of its wall. Hematoxylin and eosin. × 10.4.

(d) Higher power view of the site indicated by an arrow in (c). Fibromatous tissue with few smooth muscle fibers. The tumor showed hyaline and myxomatous degeneration in other places. × 200.

I express my gratitude to the Director, Dr. George W. Corner, and to all the personnel of the Department of Embryology of the Carnegie Institution of Washington, for their continuous help throughout the investigation.—AUTHOR.

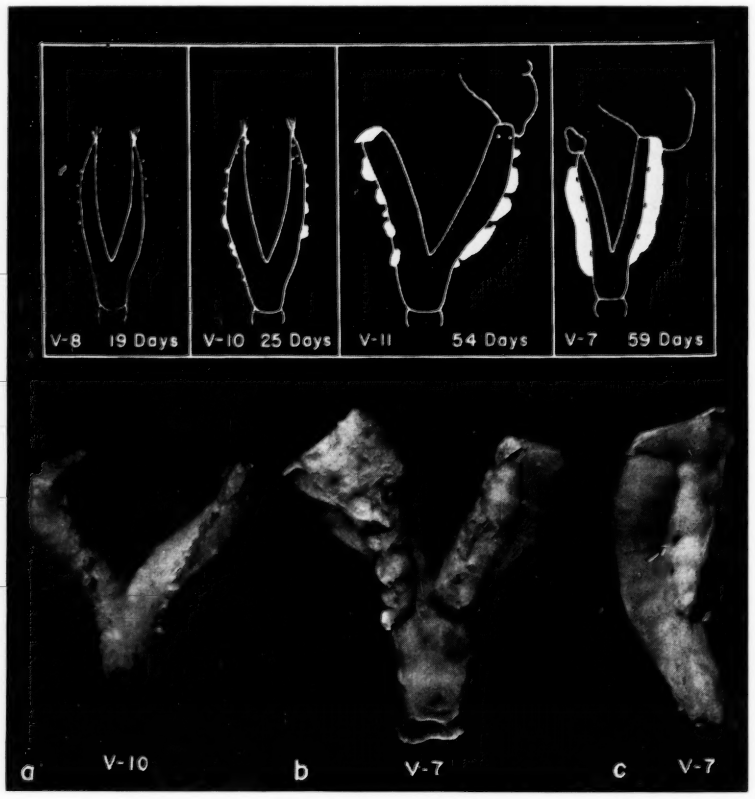

FIG. 5.—Growth of the mesometrial fibroid in hypophysectomized ovariectomized adult guinea pig.

The fibroids of the mesometrium are a good example of the growth of experimental fibroids. The miliary nodules tend to fuse together during their growth. In animal V-7, after 59 days of treatment, a macroscopic fusion of the tumor is shown. Its lobulation, the small free spaces near the uterine insertion of the mesometrium (arrow), and subsequent microscopic examination demonstrated the separate origins of the larger tumors. Natural size.

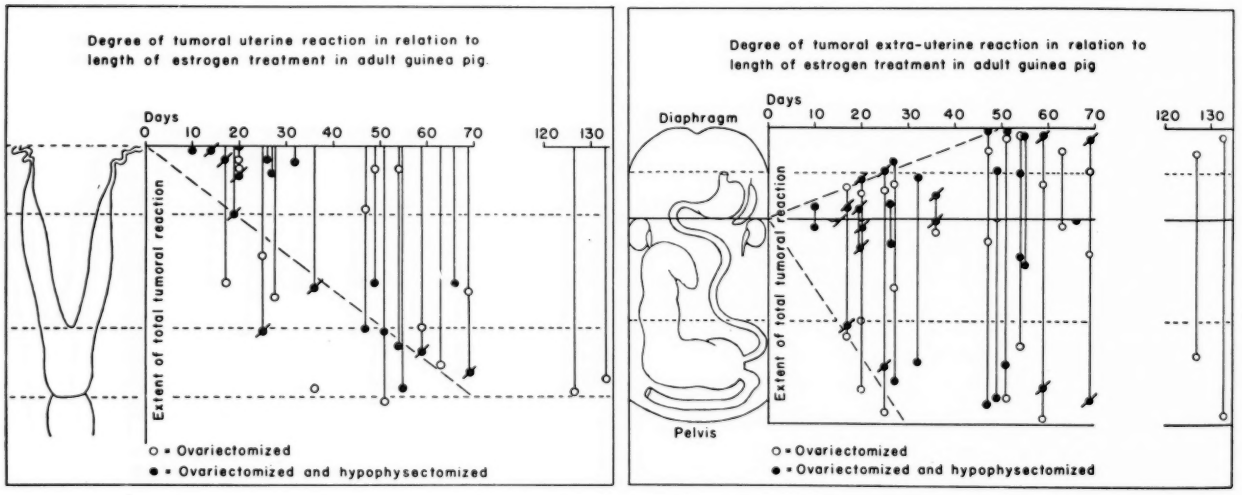
Degree of tumoral uterine reaction in relation to length of estrogen treatment in adult guinea pig.
Days
0 10 20 30 40 50 60 70 120 130
Extent of total tumoral reaction
○ = Ovariectomized
● = Ovariectomized and hypophysectomized
Degree of tumoral extra-uterine reaction in relation to length of estrogen treatment in adult guinea pig
Diaphragm
Pelvis
Days
0 10 20 30 40 50 60 70 120 130
Extent of total tumoral reaction
○ = Ovariectomized
● = Ovariectomized and hypophysectomized

FIG. 6.—This figure attempts to represent graphically the total extent of the fibroid reaction. The principal criterion for these graphs was the extent of the area involved. The picture on the right shows a horizontal dividing line passing between the stomach and kidneys. This arbitrarily indicates the usual region of origin of extragenital fibroids, from which they tend to spread, whereas genital fibroids appear first in the region of the uterine cornua. Each vertical line bearing circles above and below this horizontal line refers to one case, the vertical line indicating the extent of the tumors. The solid circles crossed by short diagonal lines represent the animals in which the removal of the anterior lobe of the hypophysis was complete. These charts represent the material listed in Tables I and II, plus 2 animals killed after 127 and 133 days of treatment.

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The Inheritance of Susceptibility to Tumors Induced in Mice

II. Tumors Induced by Methylcholanthrene in the Progeny of C3H and JK Mice*

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Various inbred strains of mice show pronounced differences in susceptibility to carcinogenic agents. Previous experiments (2) with CBA, C3H, CHI, NH, and JK mice revealed that C3H and JK mice were respectively most and least susceptible to tumors induced by subcutaneous injection of methylcholanthrene. The F_1 progeny of the C3H and JK strains have been used in additional investigation of the inheritance of this susceptibility. The experimental conditions present when the parental strains were studied have been duplicated as nearly as possible. Use of F_1 animals makes it possible to investigate sex linkage and extrachromosomal influences, as well as to ascertain induction time, survival time, and types of tumors induced.

METHODS

The reciprocal crosses of C3H and JK parents were made and the data classified accordingly (Table I). The F_1 mice were not allowed to breed and at 60 days of age received a subcutaneous injection of 1 mgm. of methylcholanthrene [1] in 0.1 cc. of sesame oil. As in the work with the parental strains, a diet of nurishmix and lettuce was used. The temperature of the room was maintained at 70° F. and the humidity at 50 to 60 per cent. The animals were examined for tumors at intervals not greater than 14 days. Sections of the tumors from one group of mice were fixed in Bouin's fluid and stained in hematoxylin and eosin. Another group was allowed to live so that the survival times might be determined. The curves of induction time

TABLE I: INDUCTION AND SURVIVAL TIMES FOR F_1 (C3H × JK) MICE INJECTED WITH METHYLCHOLANTHRENE

Hybrids	Number of mice injected	Tumors	Died without tumor after first tumor appeared	Percentage dying without tumors	Induction time in days: Mean	Induction time in days: Median	Survival time: Number	Survival time: Mean days	
Males	88	86 *	1	1.2	83.6	84	58	50.5 ± 1.2	
F_1 (C3H × JK)	187	185		0.6	86.4				51.6 ± 1.0
Females	99	99	0	0.0	87.2	85	53	52.8 ± 1.3	
Total	187	185	1				111		
Males	36	35	1		83.6				
F_1 (C3H♀ × JK♂)	90	89			87.8	85			
Females	54	54	0		90.6				
Males	52	51	0		83.4				
F_1 (JK♀ × C3H♂)	97	96			83.2	84			
Females	45	45	0		52.8				

* One male alive without a tumor 488 days after injection.

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[1] A preparation of methylcholanthrene other than that injected into the parents was used. However, Strong (4) duplicated the results reported for C3H mice (2) with the second lot of carcinogen. It is therefore unlikely that the difference in potency, if any, was appreciable.

for the F_1 were plotted on the same scale and in the same manner as for the parental strains (2).

RESULTS

Induction time is defined as the period from the injection of carcinogen to the appearance of a growing tumor. Survival time is the interval from the detection of a growing neoplasm to the death of the animal. The curves showing the induction times for

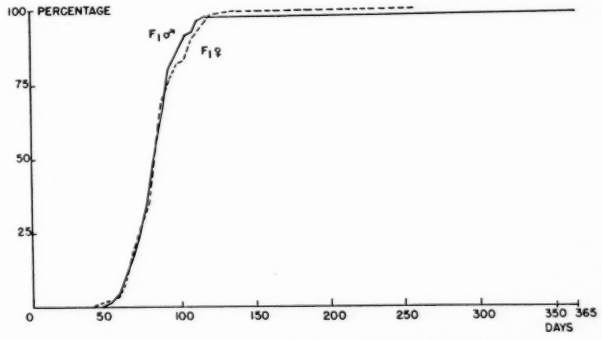

FIG. 1.—Time from injection of methylcholanthrene to appearance of tumors in F_1 males and females.

males and females (Fig. 1) are strikingly similar. There is no indication, therefore, that there is a sex difference. The median induction times are 84 and 85 days for the males and females respectively (Table I). The average survival times of the mice that developed tumors are 50.5 days for the males and 52.8 days for the females, both of which are longer than the average period of survival for the parental strains.

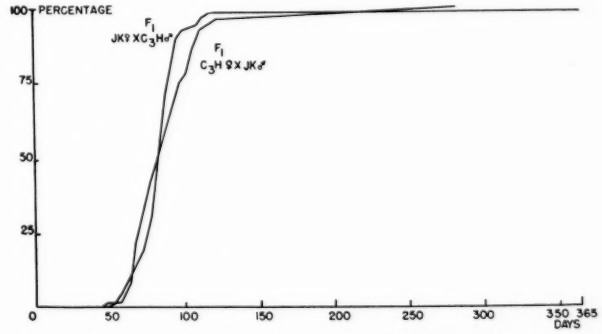

FIG. 2.—Time from injection of methylcholanthrene to appearance of tumors in the progeny of the reciprocal cross of C3H and JK mice.

The data are subdivided in order to compare the reciprocal crosses (Table I). The median induction time of the progeny of C3H females and JK males is 85 days. For the progeny of JK females and C3H males it is 84 days. The curves of induction time for the reciprocal crosses show little difference between the two groups (Fig. 2).

The curve for the F_1 values falls between the curves for the C3H and JK mice (2) and is nearer the values for the C3H than for the JK mice (Fig. 3). Superimposition of the curves plotted for the males and females of the three types of animals shows overlapping of the curves for F_1 and C3H mice only after 110 days, a value beyond the median and the mean induction times for both C3H and F_1 animals. At the present time one F_1 male has survived 488 days after injection of the carcinogen without developing a tumor.

The tumors of 50 mice were sectioned. Thirty-one (62 per cent) were spindle cell sarcomas. Five (10 per cent) were rhabdomyosarcomas, and an equal number were epidermoid carcinomas. The average induction time for spindle cell sarcoma was 90.0 days. That for rhabdomyosarcoma and epidermoid carcinoma was 78.0 days. Five mixed tumors with elements of spindle cell sarcoma and rhabdomyosarcoma appeared with average induction time of 88.0 days. Other mixed tumors found were 3 carcinoma-and-spindle cell sarcomas and one carcinoma-spindle cell sarcoma-and-rhabdomyosarcoma. Only serial sections could eliminate the possibility that some of the tumors classified as being composed of one neoplastic tissue might be

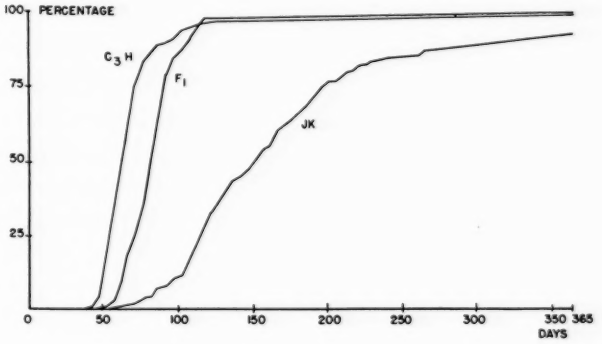

FIG. 3.—Curves of induction time for tumors in C3H and JK mice and their progeny.

mixed. There were lung tumors in 3 females and mesenteric metastases in 1 female and 1 male among 50 mice examined. Two F_1 females had mammary adenocarcinomas (5) as well as tumors at the locus of injection of methylcholanthrene.

DISCUSSION

Andervont (1) mated C3H mice with I and Y mice and found the susceptibility of the progeny to induced tumors intermediate to that of both parents. There was no evidence for extrachromosomal influence. He concluded that if there were genetic factors involved they were probably multiple. In this investigation an attempt has been made to answer several questions about the propagation of tumor susceptibility by similar methods.

As has been pointed out, the median and average induction times of the F_1 are intermediate to similar values for the parental strains. Although the curve of induction time is nearer that of the C3H than of the JK strain, the predominant type of tumor in the F_1 is spindle cell sarcoma, the same as for the JK

strain. The survival time of each class of mice is different from that of the other two, the survival time of the F_1 being greater than that of either parental strain. The longer life of tumor-bearing animals in the F_1 is probably a reflection of heterosis.

The results obtained by injecting mice of the parental strains with carcinogen (2) indicate that genetic factors for susceptibility are involved. The number and kind of genes acting are of interest. If there were only dominant genes responsible for the greater susceptibility of C3H mice, the F_1 animals should present the same curves, the same mean, and the same median as the C3H parents. This is not true (Table I and Fig. 3). If there were only recessive genes involved, the F_1 mice should have the same susceptibility as the JK parents. This also is not true. Genes for tumor susceptibility common to both strains may be detected only by outcrossing. Since the F_1 is intermediate in susceptibility, there is at least one dominant gene for tumor susceptibility in C3H mice. If it is assumed that genes are responsible and dominance is complete, the data indicate the probability that there are two or more genes responsible for tumor susceptibility and that all are not dominant and all are not recessive.

It is of interest to know whether any genes for susceptibility are linked with the sex chromosome. In C3H, JK, and F_1 mice there is no indication that susceptibility to induced tumors is sex limited in nature. In none of the data for the progeny of the reciprocal (C3H × JK) crosses is there evidence for sex linkage or a sex difference. Were there a sex linked gene or genes, there should be a difference in the susceptibility of the progeny in each reciprocal cross. The presence on the sex chromosome of a dominant gene or a dominant and also a recessive gene for susceptibility should result in F_1 females being more susceptible than F_1 males in the cross of C3H males and JK females. There is no evidence for this. Recessive sex linked genes should result in F_1 males being more susceptible than F_1 females in the cross of C3H females and JK males. This was not found to be true. Then there is no reason to believe that genes for susceptibility to induced tumors are located on the x-chromosome. The larger dose of carcinogen used may mask lesser contributors to the susceptibility effect.

It was desirable to determine whether, as in spontaneous mammary cancer (3), there is a milk influence involved in susceptibility to induced tumors. It is convenient to study this in F_1 mice, which are similar except that males of the reciprocal cross differ in having sex chromosomes derived from different parental strains. Since sex linkage should merely accentuate differences in the progeny of reciprocal crosses, no error is introduced by failure to detect a gene for tumor susceptibility on the allosomes. If there were a milk or other nonchromosomal influence, a difference in the F_1 of the respective reciprocal crosses would be expected. Since the C3H strain is the more susceptible, the reciprocal cross in which the C3H females were used should be the one in which a lesser induction time would be expected. There is no such indication either in the values of the means of the reciprocal crosses (Table I) or in a curve plotted for the induction time in these animals (Fig. 2). This is true not only when each of the two groups is considered as a whole, but also when the induction times for males and females in each cross are averaged. The difference between the lowest and highest mean in the group of males and females of the reciprocal crosses is 7.8 days.

The two mammary tumors that were found probably appeared so early as a result of the influence of methylcholanthrene, as Strong and Smith (5) have reported.

SUMMARY

1. Progeny of C3H and JK mice had average and median appearance times for tumors induced by methylcholanthrene intermediate to those of the parental strains.

2. The survival time of F_1 mice with tumors was longer than that of either parental strain.

3. The predominant type of tumor as judged by single microscopic sections was the same as that of the JK parents.

4. No evidence for linkage of susceptibility factors to the x-chromosome or for the presence of extrachromosomal influence was found.

5. The results obtained with the mice studied are compatible with the existence of more than one gene for susceptibility to induced tumors, at least one of which is dominant and at least one of which is recessive.

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Studies on Spontaneous Tumors in Guinea Pigs

III. A Chondrosarcoma of the Iliac Bone with Metastasis to the Mammary Region

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In two previous notes (3, 4) a number of spontaneous tumors arising in the gastrointestinal tract of guinea pigs have been described. The subject of this communication is a neoplasm of the iliac bone of a guinea pig with metastasis in the mammary region. It is the only example of a tumor related to cartilaginous or bony tissue found among approximately 100 collected by Papanicolaou in postmortem examination of about 7,000 guinea pigs. The large percentage of new growths observed in this stock is undoubtedly due to the fact that many of the autopsied animals were senile.

Guinea pig 5833.—The animal was a female, born Dec. 27, 1932. She was one of a group of animals treated by Papanicolaou with various extracts for experimental purposes, and had received 2 injections of extract of beef suprarenal cortex in corn oil on Oct. 13 and 16, 1933.[1] She was mated on Nov. 26, 1934, and became pregnant. The pregnancy was interrupted by a spontaneous abortion. However, she conceived immediately after the abortion and delivered 3 young on Mar. 26, 1935. On June 14, 1935, she was found to have an abscess in the neck, probably due to *Streptococcus hemolyticus,* which was prevalent at this time in the colony and caused the infection of many animals. The abscess was opened and drained. On July 19, 1935, when the animal was 2 years and 7 months old, she was found to be very weak, with hind legs paralyzed, and was killed.

Autopsy.—Two tumors were found. The first, 2×1.25 cm., occupied the anterior surface of the right iliac bone. It penetrated into the bone, which appeared somewhat swollen. The growth was pale gray in color, interspersed with dark areas, and very soft. Pieces were implanted in 2 guinea pigs. In one of these (No. 5628) a neoplasm of the adrenal, evidently of entirely different nature, developed. No tumor arose in the other (No. 5760).

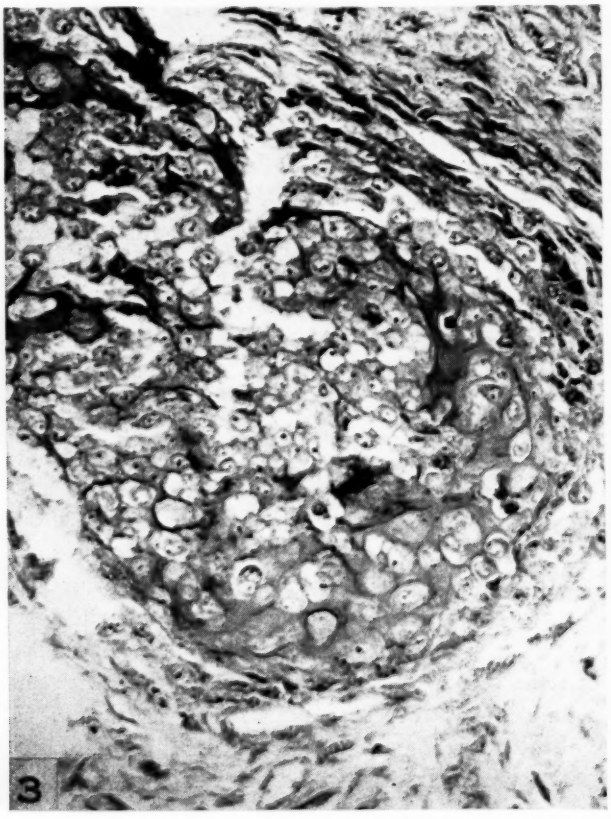
The second growth was a white, firm mass in the left mamma about 0.75×0.5 cm. The mammary tissue was dark in color but showed no hypertrophy. The other organs, including the heart, liver, spleen, adrenals, ovaries, uterus, thyroid, and hypophysis appeared normal macroscopically. The kidneys were rather large. In the apex of the left lung there was a small, firm area. Otherwise the lungs were normal.

Microscopic examination of the tumor of the iliac bone showed either smaller islands or larger areas of tumor cells surrounded by connective tissue (Fig. 1). The tumor cells were of quite uniform type. Most were round or oval (Fig. 5), others somewhat elongated or fusiform, resembling undifferentiated mesenchymal cells. The cytoplasm was basophilic and stained rather deeply. In many cells the nuclei were excentrically located. In general, the nuclei were vesicular and relatively large in comparison with the size of the cells. Their chromatin was arranged in the form of a network. Many of the nuclei had a single, large, prominent nucleolus, while others had 2 or more nucleoli.

In the islands of tumor the cells were closely packed together, more particularly near the periphery (Figs. 1 and 5). In some places there was no evidence of intercellular substance, while in others the presence of an intercellular substance could be clearly demonstrated with some stains, as, for example, Wright's stain (Figs. 3 and 4). This substance was evidently secreted or formed by the tumor cells. In some areas the intercellular substance was sparse and had a fibrillary structure (Fig. 3), while in others it became abundant and took on the aspect of the ground substance of cartilage or bone without, however, losing entirely its fibrillary structure (Fig. 4). In the latter areas the cells were more discrete and were enclosed in nests of one or several cells, each nest surrounded by a capsule. More particularly in its deeper part, embedded in the bone, the tumor had a structure closely resembling that of fibrous cartilage.

A good many mitotic figures were seen, particularly in the less differentiated parts of the growth, where the ground substance was less abundant. The islands, as well as the larger areas, were penetrated by capillaries.

[1] Animal 5652, case 6 of Paper II, with gastric and intestinal tumors, had injections of the same extract.



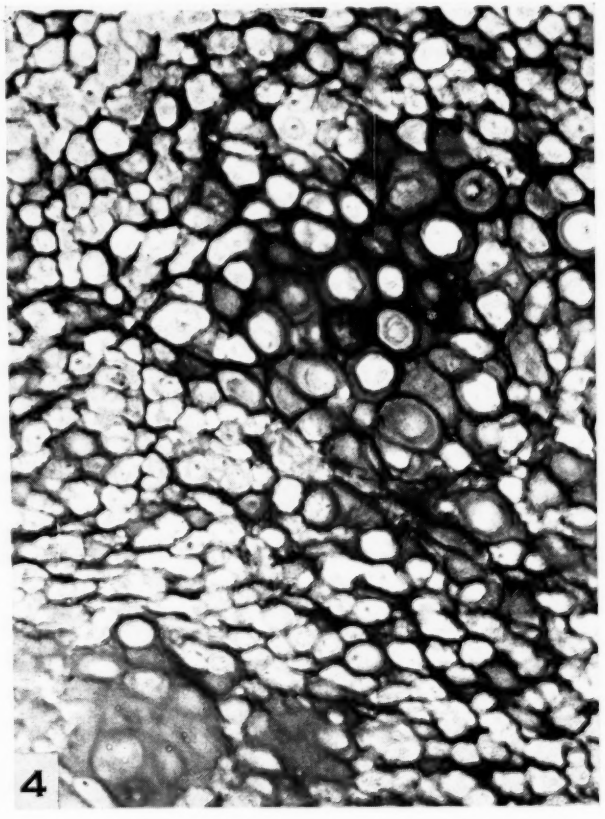
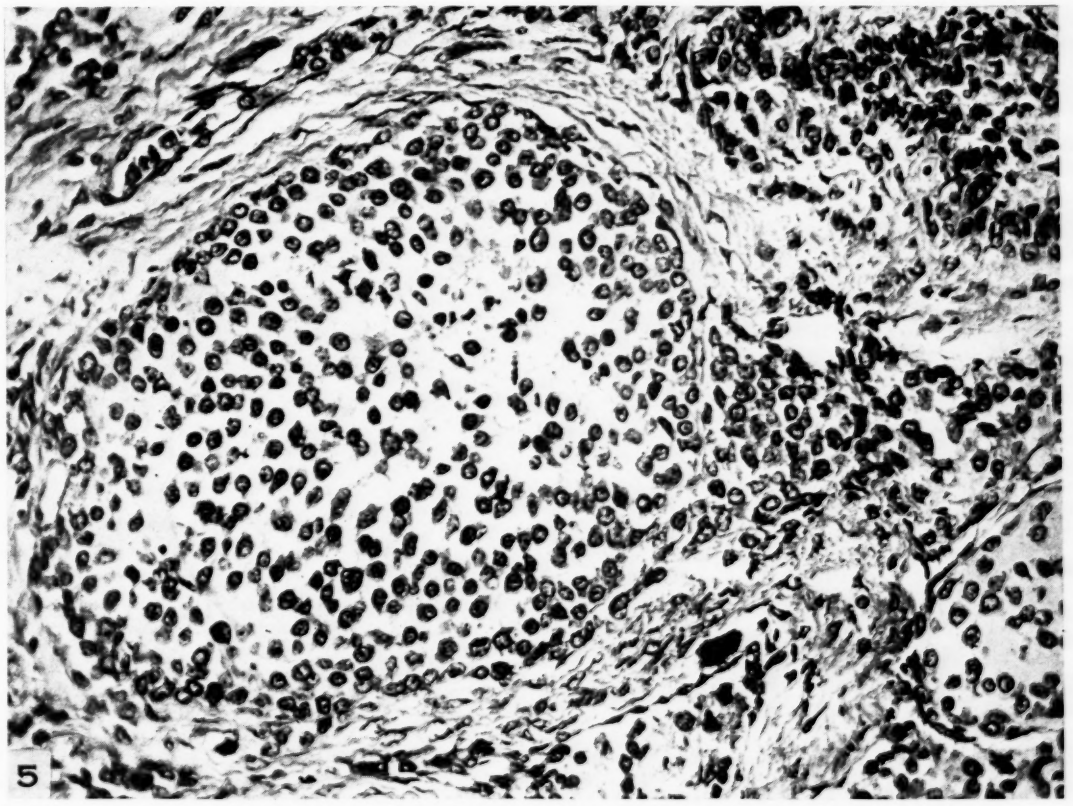


Fig. 1.—Tumor attached to ilium. Papanicolaou's stain. Mag. × 55.
Fig. 2.—Tumor adjacent to mamma. Masson's stain. Mag. × 55.
Fig. 3.—Same tumor as Fig. 1. Wright's stain. Mag. × 300.
Fig. 4.—Same as Fig. 3. Mag. × 300.



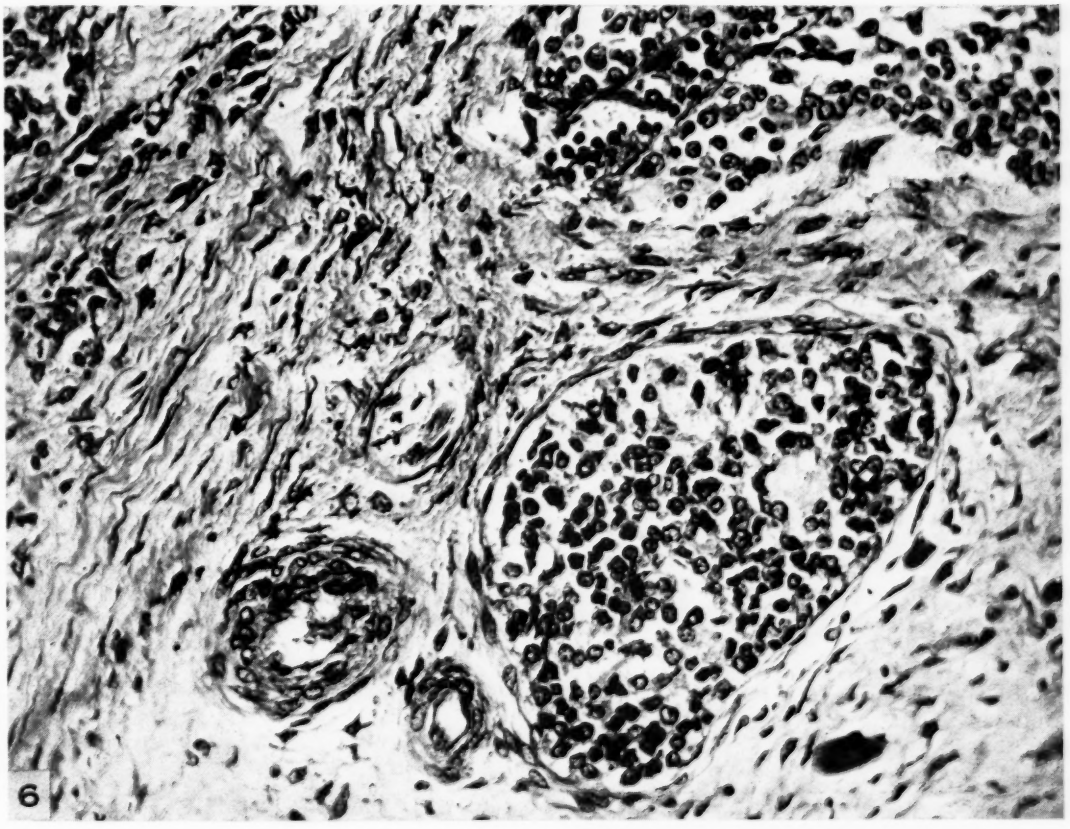


FIG. 5.—Tumor attached to ilium. Papanicolaou's stain. Mag. × 260.
FIG. 6.—Same as Fig. 5 showing tumor in endothelium-lined spaces. Mag. × 260.

They were separated by strands of connective tissue of varying thickness interspersed by arteries, distended veins, and some large lymphatics. In certain places there were extravasations of blood.

In the more superficial part of the tumor one could see a large number of striated muscle fibers embedded in loose connective tissue. In these places there were larger arteries, veins, and nerves. Many of the striated muscle fibers were dislocated and broken into small fragments that became scattered in the connective tissue. Some of the striated fibers showed dedifferentiation and degeneration, and the nuclei of the sarcoplasm were centrally placed. As the muscle fibers degenerated and broke down many of the nuclei of the sarcoplasm became scattered in the connective tissue.

In the strands of connective tissue separating the tumor masses there were distended veins and lymph vessels, containing a large number of tumor cells. Some of these vessels in which the tumor cells were very numerous and closely grouped acquired a close resemblance to the tumor islands (Fig. 6). The impression was gained that some of the islands of tumor were probably formed by the accumulation of tumor cells that had invaded the veins and lymphatics and caused their complete obliteration. A slide stained with the Foot and Foot silver technic (1) showed a complete absence of reticulum within the neoplasm.

Microscopic examination of the tumor in the region of the mammary gland.—This consisted of one mass that was divided into a number of lobules by trabeculae of connective tissue (Fig. 2). The cellular structure was similar to that of the tumor of the iliac bone except that the intercellular substance was less abundant. In the peripheral parts of the lobules the cells were closely packed, whereas in the more central parts they were more loosely arranged and separated by large empty spaces (Fig. 2). There were numerous mitotic figures. Small capillaries penetrated into the lobules. In some lobules there was a slight extravasation of blood.

The mammary tissue was pressed to one side by the tumor, and there was a fairly sharp line of demarcation between the two structures (Fig. 2). The mammary gland was not entirely atrophic, but contained many ducts and small groups of alveoli. The alveoli, as well as the ducts, were lined by cuboidal epithelium, and some contained a granular secretion in their lumina. In certain parts of the area occupied by the gland one could see some veins or lymphatics containing numerous tumor cells and similar in this respect to the vessels described in the tumor of the ilium.

Within the trabeculae separating the lobules of the tumor there were a number of ducts (Fig. 2). The lumina of some of these were entirely obliterated, while others were distended and contained abundant secretion consisting of granules and droplets. In fact the secretion appeared to be much more abundant in these ducts than in those of the mammary gland proper. The granules, as well as the droplets, were evidently secreted by the cuboidal cells lining these ducts, as they could be seen distinctly within some of the cells. A small number of ducts were detached from the connective tissue and were embedded in the lobules and surrounded by tumor cells.

Microscopic examination of the lungs.—Sections through the firm area of the lung showed chronic inflammatory changes. There was no evidence of tumor.

DISCUSSION

Tumors of mesenchymal origin have been found rather frequently in the guinea pig. Two examples of sarcoma arising from bone or cartilage have been described. Twort and Twort (5) found a malignant osteogenic tumor that involved the right kidney. It contained polygonal cells and some spindle cells and osteoclasts. In the same guinea pig there was a carcinoma of the mamma. Leader (2) described a large osteogenic sarcoma of the left femur that involved also the left tibia and fibula. It was associated with a pathological fracture of the femur. Microscopically the tumor contained cartilage and bone cells. In the lung there were gray-white nodules of metastatic osteogenic sarcoma that showed large vascular channels and again contained cells resembling those of cartilage and irregular islands of bone.

In the case described in the present paper the tumor had the nature of a chondrosarcoma and showed no evidence of calcification. The question arises which of the two tumors was primary. The fact that the histological picture was that of a chondrosarcoma might be considered as evidence that the primary tumor was in the region of bone, and the relative size of the growths is also in favor of this view. It is likely that the mammary tumor developed through metastasis in the place which, in the guinea pig, is generally occupied by a large lymph node. The ovoid shape of the neoplasm and its lobular structure would suggest the structure of a lymph node. We assume that the tumor cells were carried to this lymph node by the blood and lymphatic vessels, for, as has been noted, tumor cells were found in large numbers in the vessels adjacent to both the iliac and mammary tumors. It must be admitted, however, that there is no direct evidence to suggest which one of the two growths was primary.

There was no indication of metastasis to the lungs or to other organs, although this possibility cannot be entirely excluded. Histologically the appearance

of the tumor reported is quite different from that described by other authors in the guinea pig.

It does not appear likely that the two injections of the suprarenal extract had any influence on the inception of the neoplasm.

SUMMARY

A chondrosarcoma of the iliac bone with metastasis to the mammary region occurring in a guinea pig 2 years and 7 months old is described. Although the animal was subjected to injection with an experimental extract of the adrenal cortex, the tumor is believed to have arisen spontaneously.

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Intradermal Immunization of C3H Mice against a Sarcoma That Originated in an Animal of the Same Line

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Since the early work of Clowes and Baeslack (13) it has been observed repeatedly that mice recovering spontaneously from successfully implanted tumors usually resist reinoculation with the same neoplasm. Based on this observation a method of active immunization against tumors was devised a few years ago (6, 7). This consists of inoculating susceptible animals intradermally with a very small and carefully measured amount of finely ground tumor cell suspension. Under proper control of dosage, the resulting intradermal tumors regress in certain instances, and animals that have recovered are usually immune to reinoculation of the same neoplasm by any route.

Heretofore this method has been applied only to immunization against tumors of genetically unknown origin. In order to eliminate the possibility that the immunity thus obtained is caused by genetic differences between the tumor cells and those of the inoculated hosts, the intradermal method has been applied in the present study to the immunization of pedigreed mice of a pure inbred line against a tumor that originated in an animal of the same line. Mice of the C3H line, used in this study, have been bred by continuous brother-to-sister mating for more than 20 years (26, 27); they have thus acquired a remarkable genetic uniformity and may, for practical purposes, be considered homozygous. Should it be possible to obtain immunity in animals of the C3H line against a tumor that originated within the same line, a revision of the prevailing conception of the genetic nature of tumor immunity (4, 14, 15, 21, 23) may seem justified. Clearly, such immunity would not be caused by genetic differences between the cells of the host and those of the animal in which the tumor originated, since all mice of this line are genetically identical.

EXPERIMENTAL

Animals.—All mice were of the C3H inbred line. Animals of this line were obtained from the colony of Dr. E. W. Wallace, of the Department of Pharmacology, College of Medicine, University of Cincinnati, and breeding by brother-to-sister mating has been continued in our laboratory. Adult virgin females 6 weeks to 3 months old, and normal as well as castrated males of the same age were used for inoculations. Eight females, 8 to 9 months old, were also included. In some instances castrated, instead of normal, males were used for intradermal inoculations, since it was observed in previous studies (19) that castration increases the resistance of males to subsequent intradermal inoculation of sarcoma.

Origin and transfer of tumor.—The tumor used in this study was obtained in the following manner: Each of 4 normal adult C3H males was injected subcutaneously with 0.16 mgm. of methylcholanthrene, dissolved in 0.2 cc. of lard. One mouse (No. 1278) developed a tumor at the site of inoculation after a latent period of 65 days; the remaining 3 were discarded. The tumor was excised 10 days after its appearance, weighed, cut into small pieces, and ground thoroughly in a mortar, an 0.85 per cent solution of sodium chloride having been added under sterile conditions to obtain a 20 per cent suspension of tumor cells. The suspension was then passed through a voile filter, tested for bacterial sterility, and injected (0.5 to 0.6 cc.) under the skin of the back of 2 C3H males. All tumor cell suspensions used in our experiments were bacteriologically sterile as evidenced by negative inoculations of ordinary culture media.

Tumors appeared in both inoculated animals within the first week, and were transplanted in an essentially identical manner on the 8th day following inoculation. Twenty-four successive transfers have been made thus far, 32 normal C3H males having been used for that purpose. The rate of growth of the tumor was apparently constant throughout this study. The subcutaneous tumors grew so rapidly in all inoculated animals that on the 7th to 10th day after inoculation, when they were excised for routine transfer, they weighed 0.6 to 1.2 gm. each. A 20 per cent tumor cell suspension, prepared in the manner described above, was used for both subcutaneous and intradermal inoculations. At the time of each transfer microscopic sections of the tumor were prepared.

Histological description of the tumor.—The tumor is highly cellular. The cells are of immature type, with poorly defined, slightly eosinophilic cytoplasm. The nuclei are vesicular, of short spindle shape, with rather blunt ends; depending upon the plane of section, some appear circular or oval. The nuclear membrane is distinct. There are 1 to 3 large masses of chromatin in the center, and many fine granules of chromatin dispersed throughout the remainder of the structure. The tumor is well vascularized, the walls of most of the vessels consisting of a single layer of endothelial cells. There are also many vascular slits filled with blood and bordered only by tumor cells. The supporting stroma is scant and composed of thick and thin collagenous fibrillae that are not prominent in preparations stained with hematoxylin and eosin, but appear more clearly in sections prepared to demonstrate reticulum, and those stained by the Van Gieson and Masson trichrome methods. In sections of intradermal implants, the invasive character of the tumor is obvious. Small foci of edema, degeneration, and necrosis are present, but not abundant. The tumor was designated *sarcoma 1,* and diagnosed as a highly undifferentiated spindle cell sarcoma, probably of fibroblastic origin (Fig. 1).[1]

Technic of intradermal inoculations.—With a tuberculin syringe and a 27 gauge needle, 0.01 cc., and in certain experiments 0.02 to 0.03 cc., of the tumor suspension were injected intradermally into the freshly shaved or plucked skin in the middle of the back of the mouse. The small wheal resulting was covered immediately with a drop of collodion to prevent escape of the injected suspension.

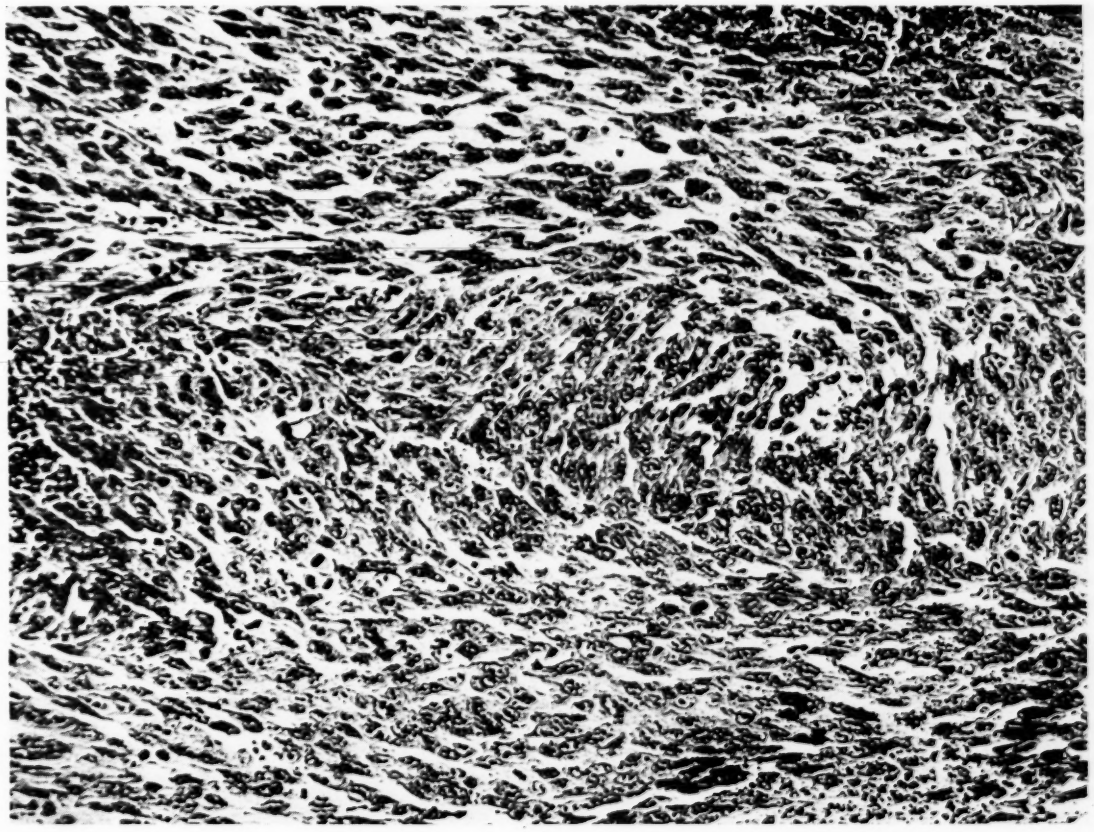

FIG. 1.—Sarcoma 1. Hematoxylin-eosin. Mag. × 202.

Susceptibility of the C3H mice to intradermal inoculations of small doses of tumor suspension.—Eighty-five mice were inoculated intradermally with 0.01 cc. of the 20 per cent tumor cell suspension (Table I). Eighty-two of these developed tumors. The 3 mice in which this first inoculation failed were reinoculated intradermally with 0.01 to 0.02 cc. of the 20 per cent suspension of sarcoma 1, and all developed sarcomas at the site of the subsequent inoculation. In addition

[1] We are indebted to Dr. Harry Goldblatt, Institute of Pathology, Western Reserve University, Cleveland, for the description of the microscopic appearance of sections of the tumor, and for the photomicrograph.

to these 85 animals, 30 mice were inoculated intradermally with 0.02 to 0.03 cc. of the 20 per cent tumor cell suspension; all 30 developed sarcomas (Table II). In both groups the intradermal tumors appeared in from 5 to 7 days, grew rapidly, and attained diameters of approximately 4 to 7 mm. and heights of about 4 to 5 mm. within 10 to 12 days following inoculation. At this time these tumors were hemispherical in shape, and the overlying skin was distinctly shiny and often of a pink color. Microscopically they were typical sarcomas, histologically identical with those resulting from subcutaneous inoculations. In 91 animals the intradermal tumors grew progressively and eventually killed their hosts in from 41 to 85 days following inoculation. In 21 mice, however, the intradermal tumors persisted only temporarily, then gradually regressed, disappearing completely in from 16 to 45 days following inoculation.

TABLE I: SUSCEPTIBILITY OF C3H MICE TO INTRADERMAL INOCULATION OF 0.01 CC. OF 20 PER CENT SUSPENSION OF SARCOMA 1

Experiment No.	Sex	Number of mice inoculated	Number of mice developing tumors	Number of mice tumors regressed
1	F	5	4	2
2	M-c *	6	6	1
3	M	1	1	0
	F	1	1	0
4	M-c	8	6	4
5	M-c	5	5	1
	F	2	2	0
6	M-c	5	5	0
7	M-c	6	6	1
8	M-c	8	8	1
9	F	3	3	0
10	M-c	5	5	1
11	M-c	1	1	0
	M	6	6	2
12	M-c	5	5	1
13	M-c	4	4	0
14	M-c	2	2	0
15	F	7	7	3
16	M	3	3	0
	F	2	2	0
Total		85	82 †	17
Takes, per cent			97	
Spontaneous regression, per cent				21

* Castrated males.

† The 3 mice in which tumors did not arise from the first inoculation were reinoculated intradermally with 0.01 to 0.02 cc. of a 20 per cent suspension of sarcoma 1, and they all developed sarcomas at the site of the subsequent inoculation.

The number of regressing tumors was substantially higher among the mice inoculated with smaller doses; thus 17 of the 85 inoculated with 0.01 cc. of the 20 per cent tumor suspension recovered from the resulting neoplasms (Table I) as compared with 4 of the 30 mice inoculated with 0.02 to 0.03 cc. of the 20 per cent suspension (Table II).

Resistance of animals that recovered to reinoculation with sarcoma.—Of the 21 animals that recovered from the intradermal tumors, one was killed accidentally, another developed a spontaneous carcinoma (Table II), and one was used for microscopic studies of the regressing intradermal tumor. The remaining 18 mice were reinoculated intradermally with 0.01 to 0.03 cc. of the 20 per cent tumor cell suspension of sarcoma 1. As control for the malignancy of the tumor cell suspension, females as well as normal and castrated males of the C3H line were inoculated intradermally at the same time and with the same tumor suspension, in equal doses except when otherwise stated (Table III, footnote §). Eleven of the 13 animals that recovered, reinoculated with 0.01 cc. of the 20 per cent tumor cell suspension, did not develop a tumor (Table III), and in 2 there arose small tumors that promptly disappeared. Fifteen control animals, inoculated simultaneously with the same dose, developed tumors and 14 of them died. Of the 5 mice reinoculated with the larger dose of 0.02 to 0.03 cc. of tumor suspension, 2 developed tumors and 3 did not. One of these tumors grew slowly but progressively, whereas the other disappeared after 70 days. Of the 10 control animals inoculated simultane-

TABLE II: SUSCEPTIBILITY OF C3H MICE TO INTRADERMAL INOCULATION OF 0.02 TO 0.03 CC. OF 20 PER CENT CELL SUSPENSION OF SARCOMA 1

Experiment No.	Sex	Number of mice inoculated	Number of mice developing tumors	Number of mice recovered
1	M	5	5	0
2	F	4	4	1
3	M-c *	3	3	1
4	F	1	1	0
5	M-c	3	3	0
	F	3	3	1 †
6	F	1	1	0
7	M-c	6	6	0
8	M-c	2	2	0
9	M-c	2	2	1
Total		30	30	4
Takes, per cent			100	
Spontaneous recovery, per cent				13

* Castrated males.

† Mouse developed a spontaneous mammary carcinoma that grew progressively, while the intradermal sarcoma completely regressed.

ously with the same dose, all developed fatal sarcomas (Table III).

A second test inoculation was performed in 12 of the 16 immunized animals that survived the first test reinoculation. None of the 4 immunized animals reinoculated with 0.01 cc. of tumor suspension developed tumors (Table IV). Of 8 animals in the same group reinoculated with the larger dose of 0.02 to 0.03 cc. of tumor suspension, one mouse developed a slowly growing but eventually fatal sarcoma, and 2 developed small tumors which later disappeared. The remainder proved refractory. In the control group of 29 mice inoculated intradermally with equal doses, all developed tumors, and only 4 recovered.

Ten animals of the immunized group were submitted to a third intradermal test reinoculation of the sarcoma. None of the 5 immunized animals reinoculated with 0.01 cc. of the 20 per cent tumor suspension developed neoplasms (Table V). Of the 5 immunized animals inoculated with 0.02 to 0.03 cc. of the 20 per cent tumor suspension, 2 developed small tumors that promptly regressed, and 1 mouse only developed a progressively growing sarcoma. Of the 21 control animals, inoculated intradermally at the same time and with equal doses of the same tumor suspension, all developed sarcomas and 19 of these died with tumors.

Immunity to subcutaneous test inoculation of the sarcoma.—Three immunized castrated C3H males (Nos. 1759, 1801, and 1815) were reinoculated subcutaneously with 0.03 cc. of a 20 per cent cell suspension of sarcoma 1. At the same time, 3 normal castrated C3H males were inoculated subcutaneously with equal amounts of the same tumor suspension. Two of the immunized animals were resistant, and one (No. 1815) developed a very slowly growing tumor that attained a diameter of approximately 3 mm. within 60 days following inoculation. The 3 control animals died with very large sarcomas in from 27 to 36 days following inoculation. Finally, 2 immunized females (Nos. 291 and 306) were reinoculated subcutaneously, each receiving the massive dose of 0.4 cc. of a 20 per cent cell suspension of sarcoma 1. Immunized females were used for reinoculation with such a massive dose of tumor because it was observed in previous studies that immunized females are more resistant than immunized males to reinoculation with sarcoma (17). At the same time, 2 normal C3H

TABLE III: RESISTANCE OF MICE THAT RECOVERED TO INTRADERMAL REINOCULATION WITH SARCOMA 1
FIRST TEST INOCULATION

20 per cent tumor suspension injected intradermally, cc.	Mice that recovered *				Normal control mice			
	Mouse No.	Sex	Interval, days †	Result	Total number of mice inoculated	Sex	Number of mice developing tumors	Number of mice dead with tumors
0.01	233	M-c ‡	42	No take	5	M-c	5	4
0.01	253	M-c	24	No take	The same control as for mouse 233			
0.01	261	M-c	28	No take	The same control as for mouse 233			
0.01	270	M-c	24	No take	1	M-c	1	1
0.01	295	M	24	No take	4	M-c	4	4
0.01	298	M	24	Take and regression	The same control as for mouse 295			
0.01	291	F	16	No take	2	F	2	2
0.01	306	F	16	No take	The same control as for mouse 291			
0.01	315	M-c	28	No take	1	M-c	1	1
0.01	1759	M-c	18	No take	2	M-c	2	2
0.01	1779	M-c	38	No take	Tumor viable §			
0.01	1801	M-c	38	No take	Tumor viable §			
0.01	1815	M-c	38	Take and regression	Tumor viable §			
0.02–0.03	1016	F	59	No take	1	M	1	1
					1	F	1	1
0.02–0.03	1243	F	22	No take	2	F	2	2
0.02–0.03	1742	M-c	64	Take and regression	6	M-c	6	6
0.02–0.03	1840	M-c	45	Take	The same control as for mouse 1742			
0.02–0.03	1794	M-c	27	No take	Tumor viable §			

* The term "recovered" in Tables III to V is used to designate those animals in which an intradermal inoculation of tumor cell suspension was followed by the formation of a tumor and the subsequent spontaneous and complete disappearance of the neoplasm.

† Interval between immunizing and test inoculations.

‡ Castrated males.

§ Control for potency of tumor only. Tumor viable, as evidenced by positive subcutaneous inoculation of the same suspension into 2 normal C3H mice.

females were inoculated subcutaneously with equal amounts of the same tumor suspension. The 2 immunized females proved refractory, whereas both control animals developed rapidly growing tumors.

days after the third intradermal test inoculation of sarcoma (Table V). Female 1016 resisted 3 successive test reinoculations of sarcoma 1, but a few days after the third reinoculation developed a progressively

TABLE IV: RESISTANCE OF MICE THAT RECOVERED TO INTRADERMAL REINOCULATION WITH SARCOMA 1
SECOND TEST INOCULATION

20 per cent tumor suspension injected intradermally, cc.	Mice that recovered				Normal control mice			
	Mouse No.	Sex	Interval, days *	Result	Total number of mice inoculated	Sex	Number of mice developing tumors	Number of mice dead with tumors
0.01	233	M-c †	48	No take	1	M-c	1	1
					6	M	6	4
0.01–0.02	253	M-c	30	Take and regression	The same control as for mouse 233			
0.01	261	M-c	34	No take	The same control as for mouse 233			
0.01	270	M-c	30	No take	5	M-c	5	4
0.01	295	M	31	No take	3	M	3	3
0.02–0.03	1016	F	60	No take	2	M-c	2	2
0.02–0.03	1243	F	63	No take	1	M	1	1
					1	F	1	1
0.02–0.03	1759	M-c	74	Take and regression	6	M-c	6	6
0.02–0.03	1779	M-c	45	No take	The same control as for mouse 1759			
0.02–0.03	1801	M-c	45	No take	The same control as for mouse 1759			
0.02–0.03	1794	M-c	27	Take	2	M-c	2	1
0.02–0.03	1815	M-c	59	No take	2	M-c	2	2

* Interval between immunizing and test inoculations.
† Castrated males.

TABLE V: RESISTANCE OF MICE THAT RECOVERED TO INTRADERMAL REINOCULATION WITH SARCOMA 1
THIRD TEST INOCULATION

20 per cent tumor suspension injected intradermally, cc.	Mice that recovered				Normal control mice			
	Mouse No.	Sex	Interval, days *	Result	Total number of mice inoculated	Sex	Number of mice developing tumors	Number of mice dead with tumors
0.01	233	M-c †	56	No take	5	M-c	5	4
0.01	261	M-c	42	No take	The same control as for mouse 233			
0.01	253	M-c	61	No take	3	M	3	3
0.01	270	M-c	38	No take	2	M-c	2	2
0.01	1815	M-c	82	No take	1	F	1	1
0.02–0.03	1016	F	106	No take ‡	6	M-c	6	6
0.02–0.03	1243	F	106	Take and regression ‡	The same control as for mouse 1016			
0.02–0.03	1759	M-c	88	No take	2	M-c	2	2
0.02–0.03	1779	M-c	62	Take	2	M-c	2	1
0.02–0.03	1801	M-c	62	Take and regression	The same control as for mouse 1779			

* Interval between immunizing and test inoculations.
† Castrated males.
‡ A spontaneous mammary carcinoma appeared in mice 1016 and 1243 a few days after the intradermal reinoculation of sarcoma.

Specificity of the acquired immunity.—It is interesting to note that immunity to sarcoma 1 did not prevent the development of spontaneous mammary carcinomas, for females 1016 and 1243 of the immunized group, both approximately 9 months of age, developed spontaneous mammary carcinomas a few

growing mammary carcinoma. Female 1243 of the immunized group developed a small cutaneous tumor at the site of the third intradermal test inoculation of sarcoma. At the same time, this mouse developed a spontaneous mammary carcinoma. A few days later, however, the cutaneous tumor disappeared completely,

whereas the spontaneous mammary carcinoma followed its usual course of slow but progressive growth until the death of the animal.

Independent development of spontaneous mammary tumors in animals inoculated with sarcoma.—Three females, 8 to 9 months old, developed spontaneous mammary carcinomas from 7 to 30 days after the initial intradermal inoculation of sarcoma 1. These two different tumors grew independently. The spontaneous and implanted tumors continued to grow progressively until the death of 2 of these animals (not included in the tables). In one female (Table II, footnote †) the spontaneous carcinoma grew steadily whereas the intradermal sarcoma persisted only temporarily, regressing spontaneously and completely 8 days before the mouse died from the very large mammary carcinoma.

DISCUSSION

The purpose of the present study was to determine whether active immunity can be produced by means of the intradermal method (6, 7) and careful dosage, in mice of a pure inbred line, against a tumor that originated in an animal of the same line. The results clearly suggest that such immunization is possible, and confirm two facts of practical importance in the study of experimental immunization against tumors: first, as has already been reported (6–9), a tumor that may be fatal when implanted subcutaneously grows only temporarily and eventually disappears in an appreciable number of instances when inoculated in very small doses into the skin of susceptible animals; secondly, it was again observed that animals in which spontaneous recovery from intradermal tumors has taken place resist reinoculation with the same neoplasm, provided the test dose is not too large.

This precaution seems to be particularly important when male animals are used for immunization, since previous studies have shown that female mice are substantially more resistant than males to inoculation of sarcoma (2, 16, 17, 18).

Theoretically, the only important difference between the present and previous (6–11) experiments on intradermal immunization lies in the origin of the tumor used for inoculation, and the relation of this tumor to the genetic constitution of the host. In previous studies on the intradermal immunization of mice, tumors of genetically unknown origin such as the Ehrlich sarcoma, Ehrlich carcinoma, or sarcoma 37, were inoculated into market mice (6, 7) or into animals of pure inbred lines (2). According to the currently prevailing conception of the genetic nature of resistance acquired against tumors (4, 14, 15, 21, 23), immunity obtained in these animals against tumors of genetically unknown origin would most likely be regarded as directed against the cells of the animal in which the tumor originated, instead of against the tumor itself. In the present study, however, animals of a pure inbred line have been immunized against a tumor that was originally induced in an animal of the same line. Such immunity could not be caused by genetic differences since all animals of this line, including the one in which the tumor originated, have a remarkable genetic uniformity, and may probably be considered homozygous. It should also be emphasized that various tumors such as sarcoma 1, induced by methylcholanthrene, and a spontaneous mammary carcinoma, may differ immunologically, although both tumors originated in animals of the same inbred line. Thus a few of our C3H females, immunized against sarcoma 1, were still susceptible to the development of a spontaneous carcinoma. These observations suggest that acquired immunity resulting from intradermal immunization is directed specifically against the particular tumor used for immunization and does not depend upon genetic differences between the cells of the tumor and those of the host.

The possibility of producing a specific immunity against tumors in susceptible animals should be stressed because hitherto so much uncontrolled experimentation has been undertaken in this field that for many years this most important problem has been close to disrepute (14). There are probably several explanations for the prevailing attitude toward the problem of experimental tumor immunity. In the first place there are, at the present time, theoretical prejudices against immunity to transplantable tumors (14, 23); secondly, inadequate methods have been used for immunization against tumors; and lastly, there seems to be much confusion in the meaning of the term "immunity" as employed by various investigators in the study of experimental cancer.

According to the prevailing conception, accepted by a large majority of students of cancer, immunity acquired against the implantation of tumors depends upon genetic differences between the tumor cells and those of the host (4, 14, 15, 21, 23). Theoretically, such immunity is not directed against the tumor, as such, but against the cells of that animal in which the tumor used for transplantation originated. This point of view seemed to find support in the fact that methods previously used for immunization against tumors, such as preliminary injection of normal organs from healthy animals (5, 28), or implantation of tumors into the tails of mice followed by amputation (1), were successful only in experiments where tumors were genetically different from the cells of inoculated hosts. These methods failed when attempts were made to produce immunity in animals of pure inbred lines

against tumors that had originated within the same strains of animals (4, 12, 15, 21). And yet failure of these methods to produce immunity, when both the host and the animal in which the tumor originated are of the same line, does not necessarily prove that the genetic theory of acquired tumor immunity is correct. The methods used for immunization might have been inadequate. Experiments reported in this paper demonstrate that intradermal immunization succeeds where methods previously used have failed.

Various other means have been used for immunization against tumors, such as injection of minute, subeffective doses of tumor, or of chemically or physically killed tumor cells, or injection of tumors from other species of animals, etc. The results were, to quote Lumsden (22) "conflicting to a surprising and bewildering degree."

Many of the conflicting results obtained by various investigators in the study of tumor immunity (28) could be explained, at least partially, by inaccurate dosage of the tumor. In view of the importance that biologists have placed on the quantitative approach to the problem of immunity in bacterial and virus diseases, it is surprising to note that accurate dosage has not been sufficiently stressed in the experimental study of tumor immunity. In most instances small pieces of tumor were inserted subcutaneously with a trocar, a procedure that practically excludes the possibility of accurate dosage. The importance, however, of accurate dosage in the study of tumor immunity cannot be overemphasized, since inoculation of too large a dose of tumor may completely obscure any differences in resistance of the inoculated animals (16, 18).

The importance of the route of inoculation also was insufficiently stressed in previous studies on immunity to tumors. Yet the same tumor may either grow temporarily and eventually regress or grow progressively and kill the host, according to the route of inoculation (24, 6, 11, 8, 9). The intradermal route seems to be the most satisfactory for immunization against neoplasms, since tumors inoculated intradermally regress spontaneously much more often than those inoculated by other routes (6, 11, 8, 9).

Important also are differences in the meaning of the term "immunity" as employed by various investigators. In most instances acquired resistance to the implantation of tumors was determined by the incidence of takes or the size of the growing tumors. Without exact dosage, however, the incidence of takes, even in uniformly susceptible animals, may be highly variable (16, 18). On the other hand, measurements, no matter how elaborate, of the size of growing tumors are not a dependable indication of immunity, since it is well known that widespread metastases may occur in animals bearing only comparatively small tumors. It is also questionable whether an animal can be called "immune" to a tumor because neutralizing or complement-fixing antibodies have been detected in its blood. The blood of animals thoroughly immunized against certain tumors may be entirely devoid of neutralizing antibodies (8–10), and, conversely, the presence of such antibodies does not necessarily indicate that the animal is immune to the neoplasm (3, 25). The term "immunity," as employed in our studies, means acquired resistance to the experimental implantation of tumor, such resistance being demonstrable only in a living host.

In conclusion it may be stated that temporary growth of an intradermally implanted tumor, followed by its spontaneous and complete regression, results in the acquisition of immunity by the host—no matter what the origin of the tumor and its genetic relation to the inoculated host. The acquired immunity is directed specifically against the tumor used for the immunizing inoculation. The nature of this immunity is still obscure. The acquired immunity is durable. Thus albino female mice of the ES line (17) immunized against sarcoma 37 were still resistant to reinoculation with the same tumor 10 months after the intradermal immunization (20).

As shown in previous studies (8–10), humoral antibodies are not necessarily responsible for the presence of such immunity. Experiments reported in this study suggest that the genetic explanation of acquired tumor immunity is probably also inadequate.

SUMMARY

One hundred and fifteen mice of the C3H inbred line were inoculated intradermally with doses varying from 0.01 to 0.03 cc. of a 20 per cent cell suspension of a sarcoma that had been originally induced by methylcholanthrene in an animal of the same line. The resulting intradermal tumors regressed spontaneously in 21 animals.

Repeated intradermal reinoculation with the same sarcoma of most of the animals that recovered were, with few exceptions, unsuccessful. Five of those that recovered were also reinoculated subcutaneously with the sarcoma, and 4 of them proved resistant. All control animals, inoculated simultaneously by the same routes and with equal amounts of the same tumor suspensions, developed sarcomas.

Since all animals, including the one in which the tumor originated, were of the same inbred line, the experiments reported in this study suggest that immunity resulting from intradermal immunization is directed specifically against the tumor used for inoculation, and does not depend upon genetic differ-

ences between the animal inoculated and the one that originally produced the neoplasm.

The theoretical significance of these observations is discussed, and the importance of accurate dosage in the study of experimental tumor immunity is emphasized.

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The Distribution of Doses of Radioactive Phosphorus in Leukemic Patients*†

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The therapeutic value of radioactive phosphorus (P^{32}), first utilized by Lawrence for the treatment of leukemia, is being rapidly established (3, 5, 7, 8, 12). In general it may be said that this radioactive isotope affords a reasonably efficacious means of radiation of leukemic cells without producing radiation sickness.

While numerous tracer studies (6) have been carried out with the aid of radioactive phosphorus, the possibility must be considered that there may be some alterations in the absorption and retention of the isotope, when given in larger amounts, due to the biological effect of the β radiation such as, for instance, a change in cell permeability. The alteration of cell permeability by radiation has been clearly established. Undoubtedly there are considerable variations in the amount of radioactive material absorbed in the different tissues because of variations in the amount of blood contained, in the degree of leukemic infiltration, in the extent of necrosis of leukemic cells, and in the extent of necrosis of normal but highly radiosensitive cells, entirely apart from the selective absorption of the tissue elements themselves.

Radioactive phosphorus [1] has been administered as Na_2HPO_4, usually in doses of 1 to 3 mc. given in approximately 300 cc. of 5 per cent glucose and 0.85 per cent saline intravenously. The amounts of total phosphorus ($P^{31} + P^{32}$) administered with each dose vary according to the specific activity of the lot used; the range is from 2 to 20 mgm. of phosphorus. The half life of P^{32} is 14.3 days. It disintegrates to sulfur, giving off β radiation that is capable of penetrating 2 to 4 mm. of tissue. As a rough comparison with more familiar types of radiation, 1 μc. retained in 1 gm. of tissue for 24 hours delivers 43 roentgen equivalents to that tissue (10).

Owing to the relatively low penetrating power of these β radiations, the effect of radiation on any given tissue depends largely upon the amount of radioactive phosphorus absorbed and retained by that tissue. Therefore it is important to know the amounts present in various tissues and fluids of the body at various times after administration of this radioactive material.

This study is based on autopsies in 10 leukemic patients performed 1 to 35 days after the last dose of radioactive phosphorus was administered. In most of the autopsies reported here the radioactive phosphorus had a high specific activity—from 0.5 to 0.14 mc. per mgm. of phosphorus. Measurements of radioactivity were carried out with the aid of a Lauritsen type electroscope or a modified Geiger counter, accurate within 10 per cent to as low as 0.001 μc. All measurements were calculated back to the date of the last injection given.

The material was obtained at autopsy by removing aliquot samples of tissue ranging from one entire organ in the case of the adrenals to 20 or 30 gm. in the case of the liver. Most of these samples were dried and ashed at 400° C. before measurement. Wet ashing was carried out on some samples and no significant variation from the dry ash method was encountered.

For convenience in discussing the data the cases have been divided into three groups: acute leukemia in adults (3 cases), acute leukemia in children (5 cases), and chronic leukemia in adults (2 cases). The deposition of P^{32} was virtually the same in all three.

Table I presents the essential data. The results are expressed in microcuries per gram of tissue and per cubic centimeter of body fluids. Our results are of much the same order of magnitude as those of Lawrence (8) and Erf (1).

Evidence that the amount of tumor may be a dominant factor in the distribution of the radioactive material is seen in our case 40 (Table I), where involved liver and lymph node gave comparable results, 0.112 and 0.112 respectively; in case 44 (Table I), where tumor tissue involving spleen, liver, and

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[1] The radioactive phosphorus was obtained through the cooperation of Professor Robley D. Evans, Massachusetts Institute of Technology; Dr. E. O. Lawrence, Crocker Radiation Laboratory; and the Harvard Cyclotron Committee.

TABLE I: RADIOACTIVITY OF TISSUES AND FLUIDS

Case No.	Days *	μc./kg.	Total dose, μc.	Microcuries per gram: Heart	Lung	Spleen	Pancreas	Liver	Intestine	Adrenal	Kidney	Uterus	Lymph nodes	Aorta	Thyroid	Muscle	Fat	Cartilage	Bone	Cortical bone	Bone marrow	Brain	Microcuries per cubic centimeter: Blood	Urine	Pericardial fluid	Spinal fluid	Bile	Pleural fluid
ACUTE LEUKEMIA IN ADULTS																												
18	5	23.6	2,965	.0465		.107		.0846			.0317			.0408					.0427										
	1	28.2																										
40	13	20.4	4,710	.079	.074	.053	.077	.112		.059	.0845	.0322	.112	.0338	.0156	.0384	.0079			.0648‡	.96‡	.0125	.0278				.052	
	6	27.7																										
51	27	46.8	6,275	.051	.076	.076	.059	.097		.065	.073		.039	.021		.041	.014	.012	.027			.009	.010			.0014	.079	
	19	49.1																										
	6	14.2																										
ACUTE LEUKEMIA IN CHILDREN																												
49	123	30.0	3,880	.03	.033	.058	.04	.058		.03	.04		.04			.02		.009	.027†		.027	.011‖	.008**			.0012	.005	.004
	107	40.0																					.008††					
	82	20.0																										
	69	50.2																										
	24	14.0																										
	22	17.5																										
	11	0.5																										
	2	2.0																										
44	38	65.0	3,680		.077	.122		.125	.0785		.1		.148			.067		.029		.086	.069		.037	.06	.0211		.0735	
	26	27.3																										
	8	27.3																										
	2	47.7																										
	24	38.1						.076			.0266		.0575	.0136		.0396		.0049	.0975†	.0324	.053	.0058	.0096			.0015	.0605	
	6	68.1																										
36	50	28.4	3,387	.0445	.053	.0705																						
	37	26.6																										
22	31	39.0	1,630			.028		.025			.031									.043	.076							
	21	6.0																										
	13	36.5																										
23	158	55.3	1,690	.0162	.027	.0535	.0229	.0365		.0056	.0382		.0493	.0124		.0256			.214‡			.00046	.0224			.00455	.0525	
	151	14.7																	.373†									
	140	50.0																	.142§									
	103	45.6																										
	35	39.8																										
CHRONIC LEUKEMIA IN ADULTS																												
8	1	35.9	2,680	.061	.0705	.082	.0366	.135			.0956		.0500	.0358	.0347				.0332†		.0987		(.142‡‡)					
																					.0332†							
9	9	33.6	2,250	.0313	.0635	.0586	.0313	.059		.0492	.0395		.0389	.0179		.022		.00856		.0356‡	.0678‡		.0116				.042	

* Number of days each administration precedes death. † Ribs. ‡ Vertebra. § Femur. ‖ Meninges. ** Right heart. †† Left heart. ‡‡ Leukocytes.

lymph nodes gave closely parallel readings, 0.122, 0.125, and 0.148 respectively; and in case 9 (Table I), spleen 0.059, liver 0.059, tumor from soft parts (intestinal nodule) 0.058, and tumor from bone marrow 0.068 mc. per gm. The time interval after administration of the last dose of radioactive material in these cases varied from 2 to 9 days.

As the time interval increased, the bone contained a relatively larger amount of radioactive phosphorus. Thus the ribs of case 23, 14 days after the administration of 901 mc., held over 10 times as much P^{32} as case 49 one day after the administration of 1,060 mc. In the short time most of our patients lived following the last dose even this was influenced by the degree of leukemic infiltration, as shown by case 23, where the amount of P^{32} retained in the tissues was highest in the rib involved in the leukemic process, less in a vertebra, which also was less involved, and least in the femur, which showed the smallest amount of leukemic infiltration. These readings were obtained 14 days after the last dose was given.

As evidence that the hepatic cells themselves as well as the leukemic cells present in the liver take up P^{32} actively, the amount present in the bile may be cited. Thus in cases 40 and 51 the amount per cubic centimeter of bile equals that per gram of spleen. However, in case 49 the bile contained relatively little. In this case there was other evidence of hepatic dysfunction.

Some tissues took up but little P^{32}, chiefly owing to the small amount of nuclear material and protoplasm in relation to total mass. Thus in case 40, fat showed one-fourteenth the concentration found in the liver and in case 51, one-eighth.

In these short term observations cortical bone did, however, show a fairly high concentration of P^{32}, as would be expected from its chemical composition.

Striking is the slight amount of radioactive material found in the brain in spite of the importance of phosphorus in its substance. This was uniformly lower than in any of the other tissues. This result may be expected in view of the low rate of metabolism of the brain. The amount in the spinal fluid was much lower than in the blood, owing to the absence of any number of cells in the spinal fluid.

Within a few hours after injection but little P^{32} remains in the blood plasma, and that circulating is practically all in the blood cells (2). Soon after injection of P^{32} the liver, spleen, lungs, kidneys, and bone contain the largest amounts of the material. These earlier results have been checked by a later series, as yet unpublished, which gives comparable results. Thus P^{32} administered to normal rodents (4, 9, 13) as well as to leukemic human subjects shows the same tendency to be deposited, at least at first, in some of those organs where leukemic infiltration is apt to occur. This localization, combined with the differential absorption by the rapidly growing leukemic cells (11), gives P^{32} a differential deposition clearly favorable to the selective irradiation of leukemic cells.

SUMMARY

The deposition of radioactive phosphorus, as determined in the tissues of 10 patients dead of leukemia, was greatest in those tissues that usually show a heavy infiltration of leukemic cells. The liver, spleen, kidneys, and bone marrow contained relatively large amounts. Slowly metabolizing tissues, as brain, fat, and cartilage, contained but little. The concentration in the bile was sometimes fairly high.

The distribution of P^{32} in human tissue is generally comparable with that obtained in rodents experimentally.

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Abstracts

Reports of Experimental Research

Carcinogenic Compounds

LASNITZKI, A., and WOODHOUSE, D. L. [Univ. of Birmingham, England] FORMATION OF HAEMOLYMPH NODES IN RATS TREATED WITH 1:2:5:6-DIBENZANTHRACENE. Nature, 150:660. 1942.

Normal rats show some hemolymph nodes, of which the most conspicuous lie near the kidneys. After injections *sub cutem* of colloidal 1,2,5,6-dibenzanthracene in a glucose-gelatin aqueous solution, many lymph nodes in various parts of the body show a red or brownish red color and, microscopically: (1) widening of lymph spaces and diminution of lymphatic tissue, (2) red corpuscles in the lymph spaces, and (3) increase of macrophages containing red corpuscles and "hemosiderin." The nodes in this condition resemble the normal hemolymph nodes. Injection of the solvent alone does not produce these changes. Experiments still in progress indicate that two other carcinogenic hydrocarbons (3,4-benzpyrene, methylcholanthrene) have the same effects, while two noncarcinogenic hydrocarbons (anthracene, phenanthrene) do not alter the appearance of the lymph nodes.—E. L. K.

LORENZ, E., and SHIMKIN, M. B. [Nat. Cancer Inst., Bethesda, Md.] DISAPPEARANCE OF INTRAVENOUSLY INJECTED METHYLCHOLANTHRENE IN MICE OF DIFFERENT SUSCEPTIBILITY TO PULMONARY TUMORS. J. Nat. Cancer Inst., 2:491-498. 1942.

Mice of strain A (highly susceptible to pulmonary tumors) and of strain C57 black (insusceptible) were injected intravenously with 0.5 mgm. of 20-methylcholanthrene dispersed in 0.5 cc. horse serum saturated with cholesterol. They were killed at various periods after injection and the lungs or whole bodies extracted for determination of the hydrocarbon content. The presence of the hydrocarbon was detected in amounts as small as 5×10^{-4} mgm. per cc. for a 1 cm. cell by a photographic method of absorption spectrum analysis in the ultraviolet.

The rate of disappearance of ether-extractable methylcholanthrene from the body was found to parallel that from the lung. In 4 days the concentration in the lung decreased to one-tenth its original value. No spectroscopic evidence of chemical change of the hydrocarbon was found, and no significant divergence was observed in the curves for the A strain or C57 black. Therefore the difference in susceptibility to pulmonary tumor shown by these strains cannot be explained by a difference in rate of elimination of methylcholanthrene.

The results indicate that intravenously injected methylcholanthrene disappears rapidly from the lungs and whole body of the mouse. Since pulmonary tumors arise within 6 weeks in strain A and within 6 to 8 months in C57 black after injection of methylcholanthrene, it seems probable that the tumors arise long after the introduction of the stimulus and long after its removal from the body.—F. L. H.

MILLER, J. A., and BAUMANN, C. A. [Univ. of Wisconsin, Madison, Wis.] THE EFFECT OF NAPHTHACENE ON THE FLUORESCENCE OF HYDROCARBONS. Cancer Research, 3: 217-222. 1943.

Details are given for the purification of carcinogenic and related hydrocarbons, for the quantitative determination of fluorescence in liquid solution, and for the photography of the fluorescence spectra of these hydrocarbons in solution and in the solid state.

One-tenth per cent of naphthacene (2,3-benzanthracene) in solid solution in 3,4-benzpyrene, 20-methylcholanthrene, 9,10-dimethyl-1,2-benzanthracene, or anthracene caused the appearance of bands in the fluorescence spectrum. These bands differed from those of either component and appeared to be characteristic of the fluorescence of naphthacene in solid hydrocarbon solution. The solid hydrocarbons and their solid solutions with naphthacene lost their ability to fluoresce upon fusion in air or *in vacuo* and immediately regained it on resolidification. 3,4-Benzpyrene when heated in liquid solution to its melting point did not lose its fluorescence.

When added to liquid solutions of the hydrocarbons, naphthacene was without effect as a fluorescence inhibitor. The so called "naphthacene effect" therefore offers no obstacle to the fluorometric determination of hydrocarbons in liquid solution.—Authors' abstract.

MILLER, J. A., and BAUMANN, C. A. [Univ. of Wisconsin, Madison, Wis.] FACTORS THAT ALTER THE FLUORESCENCE OF CERTAIN CARCINOGENS. Cancer Research, 3: 223-229. 1943.

The intensity of fluorescence in solution was measured for seven hydrocarbons in 37 solvents. In most solvents the intensity increased in the following order: napthacene < 1,2,5,6-dibenzanthracene < 1,2-benzanthracene < anthracene < 9,10-dimethyl-1,2-benzanthracene < 20-methylcholanthrene < 3,4-benzpyrene.

The fluorescence of each hydrocarbon varied with the solvent in which it was dissolved. Fluorescence was most intense in tetrahydrofurfuryl alcohol, pyridine, methyl collosolve, and dioxane; it was low in the lower alkanes, and it was zero in CS_2 or aniline.

With the exception of 1,2,5,6-dibenzanthracene, the fluorescence of these hydrocarbons was partially destroyed in solution by prolonged exposure to strong ultraviolet light. Each hydrocarbon was stable to refluxing in 10% alcoholic KOH for 1 hour.

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The most potent inhibitor of fluorescence encountered was tetranitromethane; other inhibitors studied included nitromethane, nitrobenzene, benzaldehyde, aniline, and carbon disulfide. The inhibition by tetranitromethane was irreversible; the inhibition due to CS_2 was readily reversible. $C(NO_2)_4$ inhibited the fluorescence of all compounds studied; namely, hydrocarbons, riboflavin, thiochrome, vitamin A, tissue substances, and impurities in paraffin or in ordinary solvents. It can be used in determining the "fluorometric purity" of a solvent.

The fluorescence of the unsaponifiable matter of mouse tissue was measured in 18 solvents; on a weight basis the fluorescence of these extracts was very weak as compared with that of the carcinogenic hydrocarbons. The fluorescence of the unsaponifiable matter did not vary greatly with solvent and in mixtures it was strictly additive to the fluorescence of the hydrocarbons.—Authors' abstract.

MOTTRAM, J. C., and WEIGERT, F. [Mt. Vernon Hosp., Northwood, Middlesex, England] TRANSFORMATION OF BENZPYRENE IN THE LIVING SKIN OF MICE INTO A COMPOUND SOLUBLE IN DILUTE ALKALI. Nature, 150: 635. 1942.

Benzpyrene painted on mouse skin is transformed locally into a derivative with blue fluorescence that can be found also in certain organs and in the milk of mice injected with the hydrocarbon. The fluorescence spectrum resembles that of BPX (a benzpyrene derivative found in the bile of animals injected with the hydrocarbon) but is not identical with it nor with the spectrum of the monohydroxybenzpyrene isolated from the excreta of rats injected with benzpyrene.

The derivative in the skin, which is not removed by benzene, is soluble in alkali and can be extracted with ether after acidification. It can be detected 6 hours after one painting with benzpyrene and persists for several weeks.

Microscopic study in ultraviolet light shows that this blue-fluorescing derivative is confined to the quickly proliferating cells of the malpighian layer and therefore its formation may be an important step in the production of tumors.—I. H.

NELSON, A. A., FITZHUGH, O. G., and CALVERY, H. O. [Food and Drug Administration, Washington, D. C.] LIVER TUMORS FOLLOWING CIRRHOSIS CAUSED BY SELENIUM IN RATS. Cancer Research, 3:230-236. 1943.

Eleven of 53 rats developed adenoma or low grade carcinoma in cirrhotic livers and 4 others showed decided adenomatoid hyperplasia after surviving for 18 to 24 months on diets containing 5, 7, or 10 parts per million of selenium in corn, or in wheat, or as inorganic selenide. No tumors occurred in 73 rats surviving less than 18 months, although after 3 months cirrhosis was frequent. In control rats 18 to 24 months of age, the incidence of spontaneous hepatic tumors was less than 1%. This appears to be the first report of tumors arising in experimentally cirrhotic livers after the cirrhosis had been present without tumor for a relatively long period of time.—Authors' abstract.

STEINER, P. E. [Univ. of Chicago, Chicago, Ill.] COMPARATIVE PATHOLOGY OF INDUCED TUMORS OF THE SALIVARY GLANDS. Arch. Path., 34:613-624. 1942.

By means of methylcholanthrene, 1,2,5,6-dibenzanthracene, or 3,4-benzpyrene a wide variety of cell changes, ranging from metaplasia to neoplasia, were induced in the salivary glands of mice, rats, guinea pigs, and rabbits. Metaplasia of the acinous and terminal duct cells to a squamous type of epithelium was seen as early as the 3rd day. Later epidermoid cysts, squamous cell carcinoma, and adenocarcinoma developed from the epithelium; from the connective tissue, spindle and mixed cell sarcoma developed. The mixed tumor types seen were adenocarcinosarcoma, acanthocarcinosarcoma, and acanthoadenocarcinoma, but none were composed of epithelium and cartilage as in the common human tumors. Epithelial tumors were produced in rats and guinea pigs, in which the induction of epithelial tumors is generally considered to be difficult, but no neoplasia was produced in rabbits. The ease of induction of tumors in the salivary glands is interesting in view of the rarity of such spontaneous tumors in the lower animals.—H. G. W.

STOWELL, R. E., and CRAMER, W. [Barnard Free Skin and Cancer Hosp., St. Louis, Mo.] OSTEOSIS CUTIS IN METHYLCHOLANTHRENE EPIDERMAL CARCINOGENESIS IN MICE. Arch. Dermat. & Syph., 46:276-282. 1942.

A report of 4 examples of osteosis of the skin adjacent to rather anaplastic carcinomas in Swiss mice that had been painted on a large area of the back with a 0.6% solution of 20-methylcholanthrene in benzene. Necrosis and calcification of the tissues was absent, and it is suggested that the heterotopic bone was produced by the differentiation of fibroblasts to osteogenic cells.—H. G. W.

WHITE, J., DALTON, A. J., and EDWARDS, J. E. [Nat. Cancer Inst., Bethesda, Md.] PATHOLOGY OF RAT HEPATOMA 31. J. Nat. Cancer Inst., 2:539-554. 1942.

The history and pathology of rat hepatoma 31 are presented. The tumor, a malignant hepatoma, arose in the liver of an Osborne-Mendel rat fed *p*-dimethylaminoazobenzene. It is readily transplantable in the homologous strain of rats and is now in the 16th generation. It is suitable for studies in which liver and a tumor of hepatic parenchymal cell origin are to be compared.—F. L. H.

WHITE, J., and EDWARDS, J. E. [Nat. Cancer Inst., Bethesda, Md.] EFFECT OF ORAL ADMINISTRATION OF ANILINE AND p-AMINODIMETHYLANILINE ON THE GROWTH OF THE RAT. J. Nat. Cancer Inst., 2:531-533. 1942.

This paper records the effect on the growth of the rat of the oral administration of 2 possible split products of *p*-dimethylaminoazobenzene (butter yellow); namely, aniline and *p*-aminodimethylaniline. Male Osborne-Mendel rats were raised from weaning to a weight of 75 to 80 gm. on an adequate basal diet of relatively low protein content. The transfer of these rats to a diet containing either 80 mgm. of aniline hydrochloride or 120 mgm. of *p*-aminodimethylaniline monohydrochloride per 100 gm. of the basal diet resulted in retardation of growth (failure to gain weight normally). The addition of *l*-cystine (500 mgm.) or *dl*-methionine (500 mgm.) to each 100 gm. of basal diet containing the amine stimulated growth and gave weight increases comparable to those of rats on the basal diet alone. The addition of glycine, taurine, or cysteic acid to the amine-containing diet failed to stimulate growth.—F. L. H.

Radiation

BLUM, H. F., and LIPPINCOTT, S. W. [Nat. Cancer Inst., Bethesda, Md.] ABSENCE OF HYPERVITAMINOSIS D IN MICE SUBJECTED TO ULTRAVIOLET RADIATION. J. Nat. Cancer Inst., 2:623-624. 1942.

Microscopic examination of all tissues, except those of the nervous system, from 15 mice that had received sufficient ultraviolet radiation to produce tumors of the ear revealed no lesions characteristic of hypervitaminosis D. A table giving radiant energy values and estimated amounts of the vitamin D formed, is included.—F. L. H.

Biochemistry and Nutrition

GREENSTEIN, J. P. [Nat. Cancer Inst., Bethesda, Md.] DISTRIBUTION OF ACID AND ALKALINE PHOSPHATASE IN TUMORS, NORMAL TISSUES, AND THE TISSUES OF TUMOR-BEARING RATS AND MICE. J. Nat. Cancer Inst., 2:511-524. 1942.

For various ratios of enzyme to substrate (phenylphosphate) for all the tissues studied, a linear relation, either for acid phosphatase hydrolysis (Q acid) or for alkaline phosphatase hydrolysis (Q alk.), was found to exist between the amount of hydrolysis and the nitrogen content of the tissue extract. The tissues fell into 3 groups: (1) Q (acid) > Q (alk.): Liver of the rat and mouse and the adult and fetal rabbit; muscle, skin, and spleen of the rat and mouse; and of mice, the lymph nodes, hyperplastic mammary tissue, gastric mucosa, transplanted intestinal, stomach, lung, and hepatic tumors, transplanted malignant endothelioma and melanoma, sarcoma 37, sarcoma 180, and primary induced tumor (benzpyrene). (2) Q (acid) = Q (alk.): Normal lung and bone marrow, and spontaneous mammary tumors and transplanted lymphoma 72942, all of mice. (3) Q (acid) < Q (alk.): Intestinal mucosa of the mouse; kidney of the mouse and rat; and of the rat, blood serum, Jensen sarcoma, transplanted hepatoma 31.

Mouse tumors.—Only the spontaneous mammary tumors and the lymphoma possessed alkaline phosphatase activities. The acid phosphatase activities of all were high.

Rat tumors.—The alkaline phosphatase activities were very high. Acid phosphatase activities were higher than those of mouse tumors and normal tissues.

Tumors and comparable normal tissues.—(1) Hepatic tissues: In mice the acid phosphatase was practically the same in normal and neoplastic hepatic tissues; the alkaline phosphatase was much less in the tumors than in liver. In rats both acid and alkaline phosphatase were much greater in the transplanted tumor than in normal liver. (2) Mouse mammary tissue: The acid phosphatase was nearly the same for hyperplastic mammary tissue and spontaneous tumors; the alkaline phosphatase was much higher in the latter than in the former tissue. (3) Mouse gastric and intestinal tissue: Both acid and alkaline phosphatase were much lower in the transplanted adenocarcinomas than in the respective normal mucosas. (4) Mouse lymphatic tissue: The lymphoma was lower in acid phosphatase than were the lymph nodes or bone marrow; the alkaline phosphatase of the tumor was similar to that of the nodes and less than that of the marrow.

The phosphatase activities of tissues (liver, kidney, spleen, skin, muscle) and sera of normal mice and rats and of those bearing transplanted tumors were similar.—F. L. H.

GREENSTEIN, J. P., ANDERVONT, H. B., and THOMPSON, J. W. [Nat. Cancer Inst., Bethesda, Md.] KIDNEY AND BLOOD CATALASE ACTIVITY OF TUMOR-BEARING ANIMALS. J. Nat. Cancer Inst., 2:589-594. 1942.

The liver and kidney catalase activities of the same animal were compared in normal and tumor-bearing rats and mice. The tumor-bearing animals included dilute brown mice bearing the transplanted tumors: sarcoma 37, 180, and malignant melanoma; dilute brown × I mice bearing the transplanted hepatoma 1; C3H mice bearing the transplanted hepatoma, transplanted gastric adenocarcinoma, and spontaneous mammary tumors; strain A mice bearing a primary subcutaneous tumor (benzpyrene-induced); Osborne-Mendel rats bearing the transplanted hepatoma 31; and Buffalo rats bearing the transplanted Jensen sarcoma.

The kidney catalase activity in normal mice was about half that of the livers; in normal rats it was only slightly less than that of the liver of this species. As in previous findings, the liver catalase activity of animals bearing rapidly growing tumors was much less than that of normal animals. On the other hand, with the exception of C3H mice bearing spontaneous mammary tumors, the kidney catalase activity of tumor-bearing animals was lowered relatively little so that values for catalase activity were higher for the kidney than for the liver. The decrease in catalase activity in the kidney of mice bearing spontaneous mammary tumors was greater than in the liver.

The blood catalase activity of rats bearing the Jensen sarcoma was like that of normal rats despite a lower red cell count and lower hemoglobin concentration in the blood of the former group of animals.—F. L. H.

GREENSTEIN, J. P., and STEWART, H. L. [Nat. Cancer Inst., Bethesda, Md.] NOTE ON THE ENZYMATIC ACTIVITY OF THE TRANSPLANTED ADENOCARCINOMA OF THE GLANDULAR STOMACH OF A MOUSE. J. Nat. Cancer Inst., 2:631-633. 1942.

The pepsin and thymonucleodepolymerase activity of normal gastric mucosa is compared with the enzyme activity in the transplanted adenocarcinoma of the pyloric stomach in C3H mice. The normal gastric mucosa exhibited strong pepsin activity at pH 1.39 and none at pH 5.03 as determined by hydrolysis of egg albumin and horse serum substrates. The gastric adenocarcinoma had no protease activity at either pH. Similar results were obtained in determinations of the milk-clotting activity of the two tissues.

The thymonucleodepolymerase activity of the gastric adenocarcinoma was like that of the normal gastric mucosa but considerably less than the activity of normal intestinal mucosa and intestinal adenocarcinoma.— F. L. H.

LIPPINCOTT, S. W., and MORRIS, H. P. [Nat. Cancer Inst., Bethesda, Md.] PATHOLOGIC CHANGES ASSOCIATED WITH RIBOFLAVIN DEFICIENCY IN THE MOUSE. J. Nat. Cancer Inst., 2:601-610. 1942.

C3H mice of both sexes were placed on a basal diet at weaning or at 3 months of age. A control group of 20 mice received this diet plus 10 mgm. of crystalline riboflavin. Another group was maintained on the basal diet

until the state of acute deficiency was produced and then given sufficient riboflavin to produce a state of chronic partial ariboflavinosis.

The animals of the control group grew about as well as did those on a stock diet of natural foodstuffs and showed no lesions. The young mice on the deficient diet had a maximum life span of 8 weeks. They showed retarded growth, thinned hair about the snout, ears, scapulae, and forelegs, and cracks and fissures on the snout and legs. The adult mice during a life span of 14 weeks lost weight and were similar in appearance to the young mice.

The young mice suffering a chronic partial deficiency for at least 3 months appeared smaller than the controls and exhibited more frequent and intensified cutaneous lesions. The animals assumed a hunched position on standing and on walking showed a dragging, jerky gait with mild ataxia.

The internal viscera showed no obvious lesions in mice of any group but were generally smaller than normal. Lesions were observed only in the skin, nervous system, and eyes. The skin showed atrophia and hyperkeratotic dermatosis followed occasionally by dermatitis. Myelin degeneration appeared in the spinal cord chiefly in mice in the chronic deficiency state. In the eye keratitis and occasionally the capsulolenticular type of cataract were present.—F. L. H.

MORRIS, H. P., and LIPPINCOTT, S. W. [Nat. Cancer Inst., Bethesda, Md.] PRODUCTION OF GASTRIC LESIONS IN RATS BY FASTING, PARTIAL INANITION, AND DEFICIENCY OF CERTAIN DIETARY CONSTITUENTS. J. Nat. Cancer Inst., 2:459-477. 1942.

Fourteen groups of 20 to 25 three month old Wistar strain rats were used in studying the relation of fasting, partial inanition, and specific deficiencies of protein, fat, or carbohydrate, to the production of papillomas of the forestomach or ulcers of the glandular portion of the stomach of the rat.

It was found that both types of lesions occurred after fasting and partial inanition. Choline in the drinking water increased the survival of fasting rats, but did not prevent lesions in the forestomach, and choline in 25% aqueous glucose solution prevented the formation of glandular ulcers in an otherwise fasting rat. Small amounts of any energy-producing food helped to prevent the formation of papillomas. Papillomas that formed during a 7 day period of fasting disappeared most rapidly after rats were placed on a fat-deficient diet, less rapidly on a diet deficient in carbohydrate, and least rapidly on a protein-deficient diet. Rats fed any of these diets without a preliminary fasting period did not tend to have increased numbers of papillomas, and under these conditions no papillomas became malignant. Such diets were not factors in the genesis of glandular ulcers.

Microscopic examination of material from 225 rats showed that a circulatory disturbance in the peripheral capillaries of the glandular mucosa followed by a local interference in nutrition resulted in necrosis of the acini and the overlying mucus-secreting cells, with the formation within 5 to 14 days of superficial mucosal ulcers. It was not primarily a break in continuity of the layer of mucus-secreting cells *per se* that initiated the formation of this type of ulcer.—F. L. H.

ROBERTSON, W. v.B., and KAHLER, H. [Nat. Cancer Inst., Bethesda, Md.] RIBOFLAVIN CONTENT OF TUMOR TISSUES. J. Nat. Cancer Inst., 2:595-600. 1942.

A fluorometric method, described in detail, was used in determining comparative amounts of riboflavin in primary and transplanted mouse hepatomas, and in fetal, normal, and regenerating livers of rats and mice.

Free riboflavin made up about 25% of the total amount in both hepatoma and liver. The riboflavin concentration in normal liver was 3 to 6 times that in primary or transplanted liver tumors on both a wet weight and a dry weight basis. Values for fetal liver and for tumors of 15 types ranged from 20 to 30 μgm. of riboflavin per gm. of dry tumor with the exception of mouse hepatomas, spontaneous and induced, in which the values were higher.

The total solids of 12 tumors ranged from 17 to 20% while mouse hepatomas contained 24%. The macroscopically normal livers of all tumor-bearing rats and mice had less total solids than the livers of normal controls but riboflavin was lowered only in the rats bearing hepatoma 31.—F. L. H.

WHITE, J., and EDWARDS, J. E. [Nat. Cancer Inst., Bethesda, Md.] EFFECT OF DIETARY CYSTINE ON THE DEVELOPMENT OF HEPATIC TUMORS IN RATS FED p-DIMETHYLAMINOAZOBENZENE (BUTTER YELLOW). J. Nat. Cancer Inst., 2:535-538. 1942.

Male and female Osborne-Mendel rats were raised from weaning to a weight of 75 to 86 gm. on an adequate basal diet of relatively low protein content. *p*-Dimethylaminoazobenzene was then added to the diet (60 mgm. per 100 gm. of diet). At this time, one group of rats was given a 0.5% *l*-cystine supplement (high cystine-*p*-dimethylaminoazobenzene diet) while a second group received no supplement (low cystine-*p*-dimethylaminoazobenzene diet). Two groups of rats ingesting the high and low cystine diet, respectively, with no *p*-dimethylaminoazobenzene supplements were used as controls. Results are presented only for animals that had been on the *p*-dimethylaminoazobenzene diets and were studied pathologically. The tumors of the liver were first detected by palpation through the abdominal wall. From 120 to 500 days after the beginning of the experiment the animals were killed and autopsied. The liver and other tissues were examined microscopically.

At the end of 200 days, 96.3% of the autopsied rats that had ingested the high cystine-*p*-dimethylaminoazobenzene diet proved to have developed primary hepatic carcinoma, while only 15.4% of the autopsied animals that had been on the low cystine-*p*-dimethylaminoazobenzene diet had developed such tumors. At 390 days, 61.4% of the autopsied rats of the low cystine group had developed hepatomas; and at 500 days only 60.0% of the total number in this group had developed cancer of the liver. The conclusion is reached that the major effect of the low cystine diet was to increase the latent period rather than to prevent tumor formation.—F. L. H.

Milk Influence

BITTNER, J. J. [Roscoe B. Jackson Memorial Lab., Bar Harbor, Maine] THE MILK-INFLUENCE OF BREAST TUMORS IN MICE. Science, 95:462-463. 1942.

This report presents further data on the characteristics of the active milk influence that plays an important role

in the development of spontaneous carcinoma of the mammary glands of mice.

Females were selected from the fostered C3H strain (with normal breast tumor incidence of 2%) and from the BAF_1 hybrids (C57 black ♀ × A ♂, with normal breast tumor incidence of 1%). They were given, by mouth or by subcutaneous or intraperitoneal injection, filtrates or extracts of glycerin-treated mammary tissue from lactating mice. Sixty-three experimental mice gave a mammary tumor incidence of 41%. Thirty-six mice receiving unfiltered or untreated material showed an incidence of 67%.

Previous studies have indicated that the milk influence is not inactivated by desiccation. After ultracentrifugation the active influence appeared in traces in the fat fraction and in the final supernatant fluid. It is possible that the active agent is a colloid of high molecular weight.—M. B.

BRYAN, W. R., KAHLER, H., SHIMKIN, M. B., and ANDERVONT, H. B. [Nat. Cancer Inst., Bethesda, Md.] EXTRACTION AND ULTRACENTRIFUGATION OF MAMMARY TUMOR INCITER OF MICE. J. Nat. Cancer Inst., 2:451-455. 1942.

Fresh tissue of spontaneous mouse tumors from C3H females was ground with sand, diluted with 0.9% NaCl to a 20% by volume solution, and fed in 0.2 or 0.4 cc. amounts to 46 female mice 7 to 14 days of age. The high and significant tumor incidence (45.6%) shows the presence of mammary tumor inciter in such extracts.

Milk from C3H female mice, after separation of the cream and some of the heavy curd, was centrifuged for 1 hour at 60,000 times gravity in the ultracentrifuge. The resultant fractions consisted of (1) a clear, slightly yellow, supernatant fluid, and (2) an opaque, chalky white pellet surmounted by a thin layer of clear, amber-colored, gelatinous material at its centripetal surface. After the pellet material was suspended in phosphate buffer (pH 7.0, $M/15$) and diluted to the original ultracentrifuged volume of 4.5 cc., each fraction was fed in 0.2 cc. quantities to 17 mice. Twenty-three mice served as controls. The test animals were C3H females that had been removed from their mothers (high tumor strain) and foster nursed by C57 black (low tumor strain) mothers. The incidence of tumors was: pellet suspension 82.3%, supernatant 41.2%, and controls 4.3%. The differences between groups were significant. From 79% to 96% of the tumor-inciting agent was estimated present in the pellet suspension with 4% to 21% remaining in the supernate.—F. L. H.

Immunology

GROSS, L. [Inst. Pasteur, Paris, France] A PROPOS DE LA NATURE DE L'IMMUNITÉ ANTI-NÉOPLASIQUE CHEZ LE LAPIN. [CONCERNING THE NATURE OF ANTI-TUMOR IMMUNITY IN THE RABBIT] Bull. internat. Acad. polon. d. sc. et d. lett., Cl. méd., 581-584. 1938.

The mechanism of immunity acquired against the Brown-Pearce epithelioma was investigated in male rabbits recovered from intradermal implants of this tumor. Active tumor cell suspension was injected in both testicles of the immunized animals; after an interval of 1 to 16 days, one of the testicles was removed from each animal, ground, and the resulting suspension injected into testicles of normal rabbits. It was observed that the tumor cells remaining as long as 7 days in the testicles of the immunized animals proved viable and capable of producing fatal metastases when removed and implanted in new animals. The same tumor cells lost their potency after 24 hours when placed *in vitro* in the incubator at 37° C., or after 48 hours when placed in a collodion sac in the peritoneum of a normal rabbit, or in from 1 to 3 days when injected into testicles of normal guinea pigs.—Author's abstract.

Transplantation

HOFFMAN, J. G., GOLTZ, H. L., REINHARD, M. C., and WARNER, C. G., [State Inst. for Study of Malignant Diseases, Buffalo, N. Y.] QUANTITATIVE DETERMINATION OF THE GROWTH OF A TRANSPLANTABLE MOUSE ADENOCARCINOMA. Cancer Research, 3:237-242. 1943.

The rate of growth of the tumor known as the Marsh-Simpson mouse adenocarcinoma was determined in the following manner: Suspensions of known numbers and sizes of live tumor cells were inoculated into 317 mice of the Marsh-Simpson strain, and the relation was established between the number of cells inoculated and the latent period (time in days from inoculation of the cells to palpation of the tumor). When the tumor reached approximately 0.1 cc. in size, it was removed and measured by means of a special volumetric pipette. The three values, final volume (V), volume of the cells inoculated (V_0), and time (t), were plotted and showed that the rate of growth was exponential; the exponent μ in the equation $V = V_0 e^{\mu t}$ was 0.37 per day.—Authors' abstract.

Cytology

DALTON, A. J., and EDWARDS, J. E. [Nat. Cancer Inst., Bethesda, Md.] MITOCHONDRIA AND GOLGI APPARATUS OF INDUCED AND SPONTANEOUS HEPATOMAS IN THE MOUSE. J. Nat. Cancer Inst., 2:565-575. 1942.

The mitochondria and Golgi apparatus of induced and spontaneous hepatoma cells are compared with those of normal hepatic cells and with those of livers after partial hepatectomy, bile duct ligation, and CCl_4 treatment. Mitochondria of cells of induced hepatoma are regularly fine filaments while those of the spontaneous hepatoma are spherical. The mitochondrial type characteristic of a primary hepatoma is also characteristic of the tumor in transplant. The Golgi apparatus of hepatoma cells is in the form of a variably condensed juxtanuclear network.

It is suggested that some relationship exists between the presence of this type of Golgi apparatus and the absence of exocrine secretory activity in the hepatoma cell.

The diagnostic value of the mitochondria and the Golgi apparatus with respect to neoplasms of the mouse liver is discussed.—F. L. H.

MOTTRAM, J. C. [Mt. Vernon Hosp., Northwood, Middlesex, England] OBSERVATIONS ON THE MORPHOLOGY OF TUMOUR CELLS. J. Path. & Bact., 54:511-514. 1942.

In 2 cancer cases large numbers of free malignant cells were present in aspirated pleural exudates. The cells were in good condition and many were seen dividing. Films were made of these cells for morphological study. Cell diameters ranged from 10 μ to nearly 70 μ, and the shapes of the nuclei varied considerably. Binucleate cells were seen as well as mononuclear and multinucleate giant cells.

Occasionally clusters of cells were observed that had originated presumably as the result of cells dividing but not separating. The cells of each cluster tended to be of the same morphological type. These observations are adduced as confirmatory evidence of the view previously advanced by the author as the result of the study of tar tumors of mice, that primary tumors are composed of groups of similar cells, which vary in morphology and in growth rate from group to group.—R. J. L.

Review

MURPHY, JAS. B. [Rockefeller Inst. for Med. Research, New York, N. Y.] AN ANALYSIS OF THE TRENDS IN CANCER RESEARCH. J.A.M.A., 120:107-111. 1942.

A lecture reviewing the evidence on the etiology of cancer, with the conclusion that at the present time there is no theory concerning the nature of the disease sufficiently comprehensive to be taken seriously. The experimental results offer definite support for certain conclusions. Malignancy is a universal cell potentiality in that any cell has inherent in its makeup the latent capacity for unlimited or uncontrolled growth. The degree of this potentiality for malignancy is a variable quantity for each tissue or cell type, and this degree is determined largely, if not entirely, by hereditary or predisposing factors. The malignant potentiality of a cell may be developed in the more sensitive subjects by the strain of normal physiological processes but may be set off even in resistant groups by a variety of inciting agents. The change from a normal to a malignant cell represents an alteration in the cell itself by virtue of which proliferation becomes an automatic process independent of a continuously acting provocative agent. The new property of the cell appears to develop suddenly, and this becomes a fixed character, which is transmitted to all its descendents. It may possibly be the result of a somatic mutation. There is insufficient indication at present that viruses play any important role in the general picture.—H. G. W.

Miscellaneous

REICH, C., and DUNNING, W. F. [Coll. of Physicians and Surgeons and Lenox Hill Hosp., New York, N. Y., Wayne Univ. Coll. of Med. and Detroit Inst. of Cancer Research, Detroit, Mich.] STUDIES ON THE MORPHOLOGY OF THE PERIPHERAL BLOOD OF RATS. I. NORMAL RATS. Cancer Research, 3:248-257. 1943.

The peripheral blood picture was studied in a series of 2,656 rats that included both sexes from 7 inbred strains and a group of hybrids at age levels varying from nearly full term embryos to over 400 days. Significant differences were observed in the mean hemoglobin values and the red and white cell counts in several inbred strains. Rats under 100 days of age had lower hemoglobin values and red and white cell counts with a lower percentage of polymorphonuclear leucocytes and higher percentages of lymphocytes and monocytes than older rats. Cyclic changes in hemoglobin values and red cell counts were observed in pregnant and lactating females, but the white cell counts and differential values were little affected by either pregnancy or lactation. The maximum decrease in hemoglobin value and red cell count was observed during the first 7 days after birth. The strains with the longest average life span had the highest mean white cell count and the greatest proportion of polymorphonuclear neutrophil leucocytes. The lowest mean hemoglobin value was observed in the strain of rats with a history of the highest spontaneous tumor incidence.—Authors' abstract.

DUNNING, W. F., and REICH, C. [Wayne Univ. Coll. of Med. and Detroit Inst. of Cancer Research, Detroit, Mich., and Coll. of Physicians and Surgeons and Lenox Hill Hosp., New York, N. Y.] STUDIES ON THE MORPHOLOGY OF THE PERIPHERAL BLOOD OF RATS. II. RATS INJECTED SUBCUTANEOUSLY WITH CARCINOGENIC HYDROCARBONS. Cancer Research, 3:258-265. 1943.

The peripheral blood pictures of rats were studied before and after the injection of varying localized doses of methylcholanthrene or benzpyrene. No significant changes were observed in 339 rats examined from 10 to 30 days after the subcutaneous injection of 1 to 12 mgm. of methylcholanthrene. After 40 days, however, 132 rats showed a significant decrease in the mean hemoglobin value and in the mean number of red cells and a notable increase in the percentage of monocytes. Fifty days after the injection of 1 to 6 mgm. of methylcholanthrene, 153 rats showed an average decrease of approximately 3 gm. of hemoglobin per 100 cc., with little change in the mean number of red and white cells and an increase of more than 100% in the proportion of monocytes. The latter picture persisted in rats with early tumors observed 60 to 140 days and 140 to 300 days after the injection of methylcholanthrene.

A significant increase in the mean number of red cells and in the proportion of monocytes was observed in 668 rats examined from 30 to 40 days after the subcutaneous injection of 0.2 to 3.0 mgm. of 3,4-benzpyrene. When the early benzpyrene tumors were discovered 60 to 300 days after the injection, the mean hemoglobin value had decreased significantly.—Authors' abstract.

DUNNING, W. F., and REICH, C. [Wayne Univ. Coll. of Med. and Detroit Inst. of Cancer Research, Detroit, Mich., and Coll. of Physicians and Surgeons and Lenox Hill Hosp., New York, N. Y.] STUDIES ON THE MORPHOLOGY OF THE PERIPHERAL BLOOD OF RATS. III. RATS WITH INDUCED AND TRANSPLANTED TUMORS. Cancer Research, 3:266-274. 1943.

Observations were made on the peripheral blood of rats with methylcholanthrene or benzpyrene tumors and of rats with progressively growing transplanted sarcomas of diverse origin. The peripheral blood pictures of 101 rats with advanced tumors induced by 1 to 3 mgm. of methylcholanthrene and of 122 with tumors induced by 4 to 10 mgm. of methylcholanthrene were compared with their blood pictures before injection, after injection, and when the growths were first discovered. Similar observations were made on 101 rats with advanced neoplasms induced by 0.2 to 2.0 mgm. of benzpyrene and of 89 with tumors induced by 2.0 to 3.0 mgm. of benzpyrene. In each group the continued growth of the tumor reduced the hemoglobin value and the red cell count and significantly increased the total white cell count and the percentage of polymorphonuclear neutrophil leucocytes. Observations on 431 rats bearing progressively growing transplanted sarcomas of 7 different strains showed that the progressive growth of each of these significantly reduced the hemoglobin value and increased the white cell count and the

percentage of polymorphonuclear leucocytes. The degree of anemia observed in the hosts of induced and transplanted sarcomas seemed to be related to the malignancy of the neoplasm, but the leucocytosis was apparently independent.—Authors' abstract.

TUBOI, S. [Path. Inst. d. med. Akad., Kioto.] INFLUENCE OF POTASSIUM α-NAPHTHALENE ACETATE UPON THE GROWTH OF MOUSE AND RAT CARCINOMAS. Mitt. a. d. med. Akad. zu Kioto, 32:176-188. 1941.

Mice with growing transplants of the Bashford carcinoma, and rats with growing transplants of the Sasaki-Yoshida hepatoma were treated daily with subcutaneous injections of respectively 0.5 and 1.5 cc. of a 1% solution of potassium α-naphthalene acetate. This substance (hormodin B, Boyce Thompson Institute) is effective in promoting the growth of various plant tissues. After 3 weeks of such treatment, the tumors in the treated groups were somewhat smaller than the tumors in the control animals, which had received similar daily injections of saline solution. The author concludes that α-naphthalene acetate inhibits the growth of carcinoma.—A. C.

COMPARATIVE ONCOLOGY

EDWARDS, J. E., ANDERVONT, H. B., and DALTON, A. J. [Nat. Cancer Inst., Bethesda, Md.] A TRANSPLANTABLE MALIGNANT HEMANGIOENDOTHELIOMA OF THE LIVER IN THE MOUSE. J. Nat. Cancer Inst., 2:479-490. 1942.

The original tumor, a solitary, spontaneous hemangioendothelioma found in the liver of a 15 month old male C3H mouse of the Andervont line, was successfully transplanted subcutaneously into 4 mice of the same line, 2 of these showing hepatic metastases. Transplantation has been continued from one subcutaneous transplant and from the liver metastasis in the same mouse for 5 and 4 generations respectively. The rate of growth has increased in successive transplants.

Histologically, the primary tumor had the appearance of a typical cavernous hemangioendothelioma. The transplanted tumors differed from the primary tumor only in details, such as evidence of less differentiation and of greater rate of growth of tumor cells, and the presence of numerous solid sheets of cells. The metastases were similar in general structure to the primary neoplasm; there were a few small, solid areas of cells.

Although the picture of the primary tumor suggested a benign character, the occurrence of liver metastases in 2 mice bearing transplanted tumors attested to its actual malignancy. The difficulty of correlating the expected behavior of a hemangioendothelioma with its histologic picture is stressed.—F. L. H.

EDWARDS, J. E., DALTON, A. J., and ANDERVONT, H. B. [Nat. Cancer Inst., Bethesda, Md.] PATHOLOGY OF A TRANSPLANTABLE SPONTANEOUS HEPATOMA IN A C3H MOUSE. J. Nat. Cancer Inst., 2:555-563. 1942.

Studies on a transplantable hepatoma arising spontaneously in a male mouse of the C3H strain are presented. The primary tumor and all transplants exhibited the structure of a well differentiated hepatoma. The tumor cells of the transplants contained fat and glycogen but little or no stainable alkaline phosphatase. The Golgi apparatus of the cells was similar to that in induced hepatomas of the mouse and rat and differed from that in the parenchymal cells of normal and regenerating liver. The mitochondrial pattern differed both from that in the cells of normal liver and from that in induced hepatomas.

The weight of evidence indicates that the spontaneous hepatoma of the mouse is a neoplasm, but whether it is benign or malignant is as yet an unsettled matter.—F. L. H.

LUCKÉ, B. [Univ. of Pennsylvania Sch. of Med., Philadelphia, Pa.] TUMORS OF THE NERVE SHEATHS IN FISH OF THE SNAPPER FAMILY (LUTIANIDAE). Arch. Path., 34:133-150. 1942.

Nerve sheath tumors have been found in 76 fish representative of 3 species of the snapper family. These tumors have been observed in no other species, though many fish have been examined. The growths were all subcutaneous, occurring particularly in the dorsal regions and the head, and although some were invasive none produced metastasis. They occurred in mature fish; the distribution was about equal in the sexes; and it is estimated that 0.5 to 1.0% of the fish in the Tortugas area were affected.—H. G. W.

NEUBUERGER, K. T., and DAVIS, C. L. [Univ. of Colorado Sch. of Med. and Hosps., and U. S. Dept. of Agriculture, Denver, Colo.] CEREBRAL TUMOR IN A DOG RESEMBLING HUMAN MEDULLOBLASTOMA. Cancer Research, 3:243-247. 1943.

A spontaneous cerebral tumor in a 3 year old male English bulldog is reported with clinical and anatomical findings. The neoplasm destroyed large areas of the brain substance, particularly the left basal ganglia, and obliterated most of the lateral ventricles. Histologically, it resembled human medulloblastoma. The probable origin of the growth was in the periventricular layers of immature cells. The differential diagnosis from oligodendroglioma and medulloblastoma is discussed.—Authors' abstract.

PAPANICOLAOU, G. N., and OLCOTT, C. T. [Cornell Univ. Med. Coll., New York, N. Y.] STUDIES OF SPONTANEOUS TUMORS IN GUINEA PIGS. II. TUMORS OF THE STOMACH AND INTESTINE. Arch. Path., 34:218-228. 1942.

About 100 spontaneous tumors have been observed in autopsies on approximately 7,000 guinea pigs. Tumors were rarely seen in animals less than 4 years old. Eight of the growths were found attached to the stomach or intestines. Four of the tumors of the stomach were classified as leiomyoma, 1 as a lipoma; of the tumors attached to the intestine, 1 was a liposarcoma and 2 were neurilemmoma. In addition to the tumors named, 1 animal had a tumor of the cervix, 1 a fibrosarcoma of the thoracic wall, and a third, an adenoma of the thyroid.—H. G. W.

Clinical and Pathological Reports

Etiology

MARTIN, H., and KOOP, C. E. [Memorial Hosp., New York, N. Y.] THE PRECANCEROUS MOUTH LESIONS OF AVITAMINOSIS B. Am. J. Surg., 57:195-225. 1942.

The most frequent and important factor in chronic irritation of the oral mucous membrane is probably avitaminosis B. Most patients with mouth cancer, on admission to the hospital, are suffering from avitaminosis B aggravated by the restriction of diet due to the painful mouth condition. There is considerable clinical evidence that the rest of the gastrointestinal tract is affected in these cases by the same deficiency disease, hence gastric and intestinal cancer also may be conditioned by this avitaminosis.—H. G. W.

Diagnosis—General

BUCHTEL, H. [Denver, Colo.] PERITONEOSCOPY. Rocky Mountain M. J., 39:114-115. 1942.

The role of the peritoneoscope in determining the operability of intraperitoneal neoplasms is discussed.—R. C. R.

LEVIN, A. E. [Moskauer Med. Inst., Moscow, U. S. S. R.] ZUR DIAGNOSTIK DES MAGENKREBSES. [DIAGNOSIS OF GASTRIC CANCER.] Acta Med. Scandinav., 108:448-454. 1941.

A blood reaction is described that depends upon a reduced capacity of the serum of patients with gastric cancer to absorb iodine. Thus, when Lugol's solution is added to a mixture of serum and starch solution, the orange to violet reaction indicative of starch occurs significantly earlier in cancer serum than in controls. Of 31 patients with gastric cancer, the test was positive for tumor in 30, while of 13 patients with malignant tumors in other sites, 11 gave positive tests. The blood of persons with other chronic diseases was uniformly negative. —M. J. E.

WHALEN, E. J. [Hartford, Conn.] ACCESSORY DIAGNOSTIC PROCEDURES. Connecticut, M. J., 6:177-180. 1942.

Endoscopic examination of the larynx, bronchi, and esophagus is discussed in relation to the incidence and diagnosis of cancer at these sites.—R. C. R.

Radiation—Diagnosis and Therapy

KESTON, A. S., BALL, R. P., FRANTZ, V. K., and PALMER, W. W. [Coll. of Physicians and Surgeons, Columbia Univ., New York, N. Y.] STORAGE OF RADIOACTIVE IODINE IN A METASTASIS FROM THYROID CARCINOMA. Science, 95:362-363. 1942.

The storage of radioactive iodine was studied in a patient with metastatic carcinoma from whom the primary growth (adenoma malignum) had been removed 35 years previously with no evidence of recurrence. A tracer dose of radioactive iodine was taken up more by a metastasis in the right lower femur than by the thyroid itself. Other metastases, previously irradiated with deep x-ray, failed to take up any appreciable amount of iodine.

The fact that the femur fixed a large proportion of the material suggested the possibility of using radioactive iodine therapeutically. Accordingly a dose of 10 millicuries of radioactive iodine was given. The femoral metastasis took up about 30% and the thyroid gland about 6%. Three weeks following administration the metastasis had lost about 85% of the radioactive iodine, while the thyroid still contained the original amount. A tracer dose then showed prompt uptake by the thyroid but no appreciable uptake by the femoral metastasis. This would suggest that at least the thyroid-like function of the metastasis had been impaired.—M. B.

LEUCUTIA, T. [Detroit, Mich.] FURTHER PROGRESS IN SUPERVOLTAGE ROENTGEN THERAPY. LATEST DEVELOPMENTS AND CLINICAL VALUE. Proc. Inst. Med. Chicago, 14:113. 1942.

A note on improvements in construction of the apparatus, certain technical problems, and the results obtained by the use of this form of therapy.—R. C. R.

MARTIN, C. L. [Dallas, Tex.] TREATMENT OF METASTATIC CERVICAL LYMPH NODES. Texas State J. Med., 37:236-241. 1941.

The author discontinued extensive dissection of the neck in cases of cancer of the lip or oral cavity associated with metastases to the cervical lymph nodes. Instead, he employed radium needles inserted in the primary growth and about the enlarged nodes judged to be involved by cancer. Radium was sufficient to produce regression of the primary growth but supplementary intensive roentgen radiation was required in the region of the neck. Although the number of permanent cures among the 50 patients so treated, many of whom had advanced cancers, could not be estimated so early, 26 patients appeared free from tumor for periods of 1 to 7 years.—M. J. E.

NOLAN, J. F., and ARNESON, A. N. [St. Louis, Mo.] RADIUM IN THE TREATMENT OF CORPUS CANCER. Proc. Inst. Med. Chicago, 14:148-149. 1942.

A general discussion with emphasis upon preoperative irradiation.—R. C. R.

SIMPSON, F. E., BREED, J. E., and THOMPSON, J. S. [Chicago, Ill.] RADON TREATMENT OF CANCER OF THE CERVIX UTERI. Proc. Inst. Med. Chicago, 14:18. 1942.

A report on treatment.—R. C. R.

TEAHAN, R. W., WAMMOCK, H., and WEATHERWAX, J. [Jeanes Hosp., Philadelphia, Pa.] TREATMENT OF CARCINOMA OF THE CERVIX WITH INTERSTITIAL RADIATION. J.A.M.A., 120:423-426. 1942.

Description of the treatment of cancer of the cervix with preliminary roentgen radiation and application of radium by the interstitial and intracavitary method. The number of patients (53) and the length of time involved in many of the cases, as well as the fact that many of the patients had very advanced carcinoma, make it impossible to give a picture in percentages that adequately indicates the value of the method. The immediate response to treatment has been better than with the intracavitary method previously used.—H. G. W.

TREADWELL, A. deG., LOW-BEER, B. V. A., FRIEDELL, H. L., and LAWRENCE, J. H. [Univ. of California, Berkeley, Calif.] METABOLIC STUDIES ON NEOPLASM OF BONE WITH THE AID OF RADIOACTIVE STRONTIUM. Am. J. M. Sc., 204:521-530. 1942.

Administration of radioactive strontium to 6 patients with bone tumor prior to biopsy or amputation showed

uptake chiefly by growing bone and by osteogenic tumor tissue. Various considerations seem to justify the therapeutic use of radioactive strontium in certain bone tumors. —H. G. W.

WARREN, S., and DUNLAP, C. E. [Harvard Med. Sch., Boston, Mass.] EFFECTS OF RADIATION ON NORMAL TISSUES. III. EFFECTS OF RADIATION ON THE BLOOD AND THE HEMOPOIETIC TISSUES, INCLUDING THE SPLEEN, THE THYMUS AND THE LYMPH NODES. Arch. Path., 34:562-608. 1942.

An extensive review.—H. G. W.

Nervous System

BAKER, A. B. [Univ. of Minnesota, Minneapolis, Minn.] METASTATIC TUMORS OF THE NERVOUS SYSTEM. Arch. Path., 34:495-537. 1942.

A clinicopathologic study of 114 tumors metastatic to the nervous system is reported. Ninety-two tumors were investigated histologically. This type of lesion is much more frequent than is generally suspected. Of all intracranial neoplasms studied at the University of Minnesota, 17.9% were metastatic. The majority of intracranial metastases are probably hematogenous. The most common primary sources of the metastases in this series were the lung, breast, gastrointestinal tract, and kidney. Histologically, tumor cells could be seen within the arteries of the brain. These cells by their growth obliterated the vessels and produced the early neoplastic lesions. In most cases there was a remarkable resemblance between the primary lesion and the metastases in the nervous system. Cerebral or cerebellar metastases often produced softening of the surrounding brain tissue but usually stimulated little or no glial reaction. Toxic changes were not observed in tissues not directly involved by the tumor elements, and remote brain changes were not seen. When meningeal metastases secondarily invaded the brain it was usually along the perivascular spaces of the meningeal vessels as they penetrated the organ.—H. G. W.

JENKINSON, E. L., and OLDBERG, E. [St. Luke's Hosp., Chicago, Ill.] RESULTS OF CONSERVATIVE TREATMENT IN CERTAIN CEREBRAL GLIOMAS. Am. J. Roentgenol., 47:50 55. 1942.

Cerebral gliomas present a poor prognosis regardless of the treatment. Attempts at radical removal carry a high operative mortality, seldom if ever result in cure, and often involve serious impairment of cerebral function. The authors advise conservative management, usually involving decompression followed by roentgen therapy, as the best means of relieving distress and prolonging the useful life of the patient. Heavy roentgen therapy often brings symptomatic relief even in this unfavorable group of tumors. Conservative treatment generally offers some two years of survival with comparative comfort and preservation of faculties.

In discussion, Dr. Gilbert Horrax presents the case for radical surgery in cases of glioma. In his experience, radiation therapy is of questionable benefit.—C. E. D.

SIRIS, J. H., and ANGRIST, A. [Queen's Gen. Hosp., Jamaica, N. Y.] CHONDROBLASTIC MENINGIOMAS. Am. J. Surg., 57:162-167. 1942.

A case of chondroblastic meningioma, the 26th recorded in the literature, is presented with a discussion of the origin and the clinical aspects of the disease.—H. G. W.

Breast

BRANSFIELD, J. W., and CASTIGLIANO, S. G. [Philadelphia, Pa.] THE INADEQUACY OF SIMPLE MASTECTOMY IN OPERABLE CANCER OF THE BREAST. Am. J. Roentgenol., 47:748-758. 1942.

The treatment of cancer of the breast is reviewed from the early attempts at excision up to the present. A well organized and documented argument is presented in favor of radical surgery in all operable cases.—C. E. D.

DAWSON, E. K., and HARVEY, W. F. [Royal Coll. of Physicians, Edinburgh, Scotland] MACRO- AND MICRO-DIAGNOSIS OF CANCER. A LABORATORY SURVEY OF ROUTINE MAMMARY LESIONS. Edinburgh M. J., 49:401-408. 1942.

By correlation of the macroscopic and microscopic aspects of mammary lesions it is shown that the pathologist can make a very accurate diagnosis of the condition, whether malignant or benign tumor or nonneoplastic, by examination by sight and touch of a single plane of section across the operative material. Inability thus to diagnose the condition occurred only once in a series of 181 benign and malignant tumors.

In this comparatively small series of breast cases the following facts were noted: in persons over 65 years of age, 100% of all lesions received for examination were malignant; over 60 years, 95%; over 55 years, 92%; and over 50 years, 85%. Under 30 years 100% were benign conditions, either tumor or some other lesion; under 40 years 84% were benign, while between 40 and 50 years 60% were benign and 40% malignant.—A. H.

FOOT, N. C. [Cornell Univ. Medical Coll. and New York Hosp., New York, N. Y.] A SIMPLER CLASSIFICATION OF MAMMARY TUMORS. Arch. Path., 33:905-916. 1942.

Tumors of the breast can be readily classified according to their histogenetic characteristics, and such a classification is devised. It is particularly recommended that epithelial tumors of the breast be arrayed in three groups only: duct cell, canalicular, and acinous.—H. G. W.

Female Genital Tract

BROWN, W. E., and TOLLMAN, J. P. [Univ. of Nebraska Col. of Med., Omaha, Neb.] CARCINOMA OF UTERUS. Nebraska State M. J., 26:363-366. 1941.

A discussion from the clinicopathological standpoint of 4 cases of cancer of the uterus.—M. J. E.

KIRTZ, L. P., and PAREIRA, M. D. [Ellis Fischel State Cancer Hosp., Columbia, Mo.] PREGNANCY COMPLICATED BY CARCINOMA OF THE CERVIX. REPORT OF A CASE. J. Missouri M. A., 38:324-326. 1941.

During the latter third of pregnancy, intracavity radium therapy was administered for carcinoma of the cervix. After this treatment, delivery of a normal child by cesarian section, and subsequent roentgen therapy, there was an apparently complete regression of the tumor.—M. J. E.

Male Genital Tract

DOCKERTY, M. B., and PRIESTLEY, J. T. [Mayo Clinic, Rochester, Minn.] LYMPHOSARCOMA OF THE TESTIS (REPORT OF FOUR NEW CASES). J. Urol., 48:514-523. 1942.

Among 400 instances of malignant growths of the testicle seen at the Mayo Clinic, only 4 were lympho-

sarcoma. The tumor is frequently bilateral and sometimes associated with sarcoma of the cutis. It is probably metastatic in nature and constitutes the majority of malignant neoplasms which spread to involve the testicle.—H. G. W.

KIEFER, J. H. [Chicago, Ill.] TUMORS OF THE TESTICLE. HORMONE DETERMINATION AND DIAGNOSIS AND MANAGEMENT. Proc. Inst. Med. Chicago, 13:299. 1941.

Urinary hormone excretion was investigated in 13 patients with tumor of the testicle and in 6 patients with nonneoplastic lesions of this organ. The test was accurate in determining whether or not a given swelling was neoplastic.—R. C. R.

SCHULTE, W. G. [Salt Lake City, Utah] TUMORS OF THE SPERMATIC CORD. Rocky Mountain M. J., 39:189-192. 1942.

A case report with discussion and a brief review of the literature.—R. C. R.

TENENBAUM, J. [New York, N. Y.] LYMPHOSARCOMA OF PROSTATE AND EPIDIDYMIS: CASE REPORT. J. Urol., 48:113-118. 1942.

A rare case is reported.—H. G. W.

Urinary System—Male and Female

ATKINSON, R. C. [Stanford Univ. Sch. of Med., San Francisco, Calif.] SKIN METASTASES FROM BLADDER TUMORS. J. Urol., 48:350-356. 1942.

A case report.—H. G. W.

DICK, V. S. [Lahey Clinic, Boston, Mass.] PAPILLOMA OF THE RENAL PELVIS ASSOCIATED WITH AN EARLY RENAL CELL CARCINOMA. Lahey Clinic Bull., 2:231-234. 1942.

Pyelographic examination in a 51 year old man revealed a filling defect in the right renal pelvis. Right nephrectomy and ureterectomy were done. A benign polyp was found in the pelvis and a 0.5 cm. early renal cell carcinoma in the cortex of the kidney. The tumors were considered to be independent primary growths.—C. E. D.

FOULDS, G. S. AN UNUSUAL KIDNEY TUMOR: MALIGNANT PAPILLARY CYSTADENOMA AND PAPILLARY CARCINOMA WITH CLEAR CELLS. J. Urol., 48:131-135. 1942.

A renal tumor containing elements of malignant papillary cystadenoma and papillary carcinoma with clear cells is reported. This case, with others that have been recorded, illustrates the transition from renal adenoma to malignant cystadenoma and eventually, in some instances, to papillary carcinoma with clear cells.—H. G. W.

HERGER, C. C., and SAUER, H. R. [State. Inst. for the Study of Malignant Diseases, Buffalo, N. Y.] LARGE BONE METASTASES FROM CARCINOMA OF THE BLADDER. Am. J. Surg., 57:29-37. 1942.

Metastatic involvement of the skeleton is common in all cases of carcinoma of the bladder in which widespread metastases occur, especially if the tumor is of the papillary type. However, cases with large bone metastases dominating the clinical picture are rare; four such cases are reported.—H. G. W.

LANGWORTHY, H. T., and DREXLER, L. S. [Cumberland Hosp., Brooklyn, N. Y.] CARCINOMA IN CROSSED RENAL ECTOPIA. J. Urol., 47:776-783. 1942.

Renal ectopia is itself an unusual condition, and no instance of carcinoma in ectopic kidneys, previous to the 2 cases in this report, has been found in the literature.—H. G. W.

PRINCE, C. L. [Univ. of Virginia Sch. of Med., Charlottesville, Va.]. PRIMARY ANGIO-ENDOTHELIOMA OF THE KIDNEY: REPORT OF A CASE AND BRIEF REVIEW. J. Urol., 47:787-792. 1942.

A case is added to the few such tumors already reported.—H. G. W.

SAUER, H. R. [N. Y. State Inst. for the Study of Malignant Diseases, Buffalo, N. Y.] CASE OF LARGE BONE METASTASIS FROM CARCINOMA OF THE URETER COMPLICATED BY CONGENITAL GIANT HYDRONEPHROSIS. J. Urol., 48:467-470. 1942.

A case is reported.—H. G. W.

SCHILLER, W. [Cook County Hosp., Chicago, Ill.] HISTOGENESIS OF THE SO-CALLED GRAWITZ TUMOR. Arch. Path., 33:879-904. 1942.

Embryological considerations, the widely separated origin of the metanephros and the adrenal cortex, as well as the numerous renal cortical adenomas of adrenal cell character found far from the upper pole of the kidney, speak against the old theory of misplaced adrenal cells as the origin of the Grawitz tumors. The presence of characteristic hyaline droplets and hemoglobin is functional evidence of the renal tubule character of the tumor cells. The histologic morphologic findings tend to support the conception that in early fetal times, when the mesenchyma is still pleuripotent, the matrix of the renal and of the adrenal cortex possesses the potency to differentiate into both renal and adrenal tissue, this potency being extinguished during fetal development. The awakening of latent potentialities occurs frequently during neoplastic proliferation of renal character and may produce tissues of a hybrid type; *i.e.*, cells with combined renal and adrenal characteristics. This can serve as an explanation for Grawitz tumors with cystic and papillary structures corresponding to tumors originating from renal cortical tubules but containing polyhedral fat-storing cells resembling cells of the adrenal cortex. —H. G. W.

Oral Cavity and Upper Respiratory Tract

DUNCAN, G. W., and DANIEL, R. A., Jr. [Vanderbilt Univ., Nashville, Tenn.] CYSTIC TUMOR OF THE TONGUE. REPORT OF AN UNUSUAL CASE. Arch. Surg., 44:164-169. 1942.

In this case squamous and columnar cell epithelium and mucous membrane with the components of the stomach wall were included in the cyst. An embryonic cell rest was considered in attempting to explain the etiology.—R. C. R.

Intrathoracic Tumors—Lungs—Pleura

QUINLAND, W. S. [Meharry Med. Coll., Nashville, Tenn.] BRONCHOGENIC CARCINOMA. REPORT OF THREE CASES IN NEGROES. South. M. J., 35:729-732. 1942.

Carcinoma of the lung has not been detected in a certain colored hospital in a single case in 19 years, but 3 examples in colored men are reported from a mixed service in Chicago.—H. G. W.

Heart

HERBUT, P. A., and MAISEL, A. L. [Jefferson Med. Coll. Hosp., Philadelphia, Pa.] SECONDARY TUMORS OF THE HEART. Arch. Path., 34:358-364. 1942.

Among 4,050 necropsies, there were 640 cases of cancer. In 35 of the cancer cases the heart was secondarily involved but in no instance was the cardiac metastasis diagnosed before death.—H. G. W.

Gastrointestinal Tract

O'DONOGHUE, J. B., and JACOBS, M. B. [Cook County Hosp., Chicago, Ill.] LYMPHOSARCOMA OF THE STOMACH. Proc. Inst. Med. Chicago, 14:108-109. 1942.

Five hundred gastric lymphosarcomas have been reported since the original description of this condition in 1816. In the records of the Cook County Hospital the incidence of this disease is about one in every 60 cases of carcinoma of the stomach. Pathology and treatment are briefly discussed—R. C. R.

Liver

MORTON, H. B. [Lincoln General Hosp., Lincoln, Neb.] LARGE PEDUNCULATED CAVERNOUS HEMANGIOMA OF THE LIVER. CASE REPORT. Am. J. Surg., 56:673-675. 1942.

A report of a tumor measuring 20 × 15 × 10 cm. successfully removed by operation.—H. G. W.

TOMLINSON, W. J., and WOLFF, E. [Laird Mem. Hosp., Montgomery, W. Va.] PRIMARY LIVER-CELL CARCINOMA IN INFANCY. REPORT OF TWO CASES, ONE SHOWING CALCIFICATION. Am. J. Clin. Path., 12:321-327. 1942.

A report of 2 cases of liver carcinoma, one with calcification, in 18 month old infants.—H. G. W.

Bone and Bone Marrow

ATSATT, R. F. [Orthopedic Division, Santa Barbara Clinic, Santa Barbara, Calif.] OSTEOGENIC SARCOMA AS THE CONCOMITANT OF AN INDUSTRIAL ACCIDENT. Am. J. Surg., 57:143-146. 1942.

A cowboy received an injury to the fibula, and a sarcoma developed within a year at the site of the injury.—H. G. W.

CHRISTOPHER, F. [Evanston Hosp., Evanston, Ill.] TOTAL EXCISION OF THE SCAPULA FOR RETICULUM CELL SARCOMA. Proc. Inst. Med. Chicago, 13:309. 1941.

A case report.—R. C. R.

CONLEY, A. H., and MILLER, D. S. [Cook County Hosp., Chicago, Ill.] NEURILEMMOMA OF BONE. A CASE REPORT. J. Bone & Joint Surg., 24:684-689. 1942.

Report of a tumor located in the first sacral vertebra. The growth was benign and was removed successfully by operation.—H. G. W.

LINDEBOOM, G. A., and MULDER, H. J. [Wilhelmina Gasthuis, Amsterdam, Holland] MULTIPLES MYELOM MIT LEUKÄMISCHEM BLUTBILD UND DEGENERATIVEN RÜCKENMARKSÄNDERUNGEN. [MULTIPLE MYELOMA ASSOCIATED WITH LEUKEMIC MANIFESTATIONS AND SPINAL CORD DEGENERATION] Acta Med. Scandinav., 108:363-373. 1941.

Leukemic manifestations in the blood of patients with myeloma are unusual. In the cases recorded, death resulted from urinary complications secondary to a transverse lesion in the lower dorsal cord. The presence of degenerative lesions in the nervous system was proved at necropsy to be secondary to an extradural focus of myeloma involving the vertebrae. The peripheral leukocytes numbered as high as 45,000, of which 42% appeared to be abnormal plasma cells. Myeloid deposits were likewise found in the liver.—M. J. E.

O'DONOGHUE, D. H. [Oklahoma City, Okla.] A CASE OF XANTHOMA OF THE KNEE JOINT. J. Bone & Joint Surg., 24:940-941. 1942.

Report of the 42nd case on record.—H. G. W.

SLOAT, J. I., and PETERSON, L. T. [Walter Reed Hosp., Washington, D. C.] EWING'S TUMOR OF THE SACRUM. FIVE YEAR SURVIVAL AFTER IRRADIATION. J. A. M. A., 119:1499-1500. 1942.

Report of a case with 5 year survival following biopsy and intensive irradiation.—H. G. W.

Leukemia, Lymphosarcoma, Hodgkin's Disease

EVENSEN, O. K., and SCHARTUM-HANSEN, H. [Drammen Hosp., Drammen, Norway] THE SYMPTOMATOLOGY OF ALEUKEMIC PARAMYELOBLASTIC LEUKEMIA. Acta Med. Scandinav., 107:227-281. 1941.

The unusual disease described is a chronic, relatively mild type of leukemia, occurring at any age, and, because of the inconclusive nature of the blood smear and clinical evidence, difficult to diagnose without recourse to a sternal puncture. The authors report their observations on 22 cases. Fatigue, dependent upon the associated anemia, and pain in the skeletal system or joints are the common symptoms. Examination of the peripheral blood discloses, in addition to the secondary anemia and thrombocytopenia, a normal or subnormal number of leukocytes. An apparent increase in a mononuclear type of cell depends upon the presence of a considerable number of paramyeloblasts. Corroborative evidence is found in the homogeneous leukemic sternal marrow present in all patients. Enlargement of the spleen, lymph nodes, or liver is not a prominent feature of the disease. Spontaneous remissions occur commonly, but the process eventually proves fatal.—M. J. E.

HELD, I. W., and CHASNOFF, J. [Beth Israel Hosp., New York, N. Y.] GIANT FOLLICULAR LYMPHOBLASTOMA (GIANT LYMPH FOLLICLE HYPERPLASIA). Am. J. M. Sc., 204:232-239. 1942.

The clinical course of the disease is outlined; it may terminate with the picture of lymphosarcoma, lymphatic leukemia, or Hodgkin's disease. Two cases are reported.—H. G. W.

HU, C. H., and PAI, H. C. [Peiping Union Med. Coll., Peiping, China] USE OF THE SUPRAVITAL STAINING TECHNIC IN THE STUDY OF TUMORS OF THE LYMPHOSARCOMA GROUP. Arch. Path., 34:106-116. 1942.

The cells of 3 tumors of the lymphosarcoma group were studied by means of the supravital staining technic in conjunction with the ordinary histologic methods. Certain features of the tumor cells, inconspicuous or unnoticeable in the ordinary microscopic sections, are rendered prominent by this technic and it is therefore suggested that supravital staining be used in combination with the ordinary histologic methods in the study of tumors of the lymph nodes and bone marrow.—H. G. W.

KRUMBHAAR, E. B., and STENGEL, A. [Univ. of Pennsylvania Sch. of Med., Philadelphia, Pa.] THE SPLEEN IN THE LEUKEMIAS. Arch. Path., 34:117-132. 1942.

An analysis of 209 cases of leukemia showed an almost equal division into the 4 types; namely, acute and chronic

lymphatic, and acute and chronic myelogenous. In general, patients with chronic forms of the disease have larger spleens than those with acute forms, and those dying in aleukemic stages tend to have smaller spleens than those in whom pronounced leukocytosis persists to the end. If the neoplastic nature of all true leukemias is accepted lymphosarcoma can be regarded as essentially the same disease as lymphatic leukemia; namely, a neoplasm of the lymphocyte.—H. G. W.

SYMMERS, D. [Dept. of Hosps., City of New York, N. Y.] CLINICAL SIGNIFICANCE OF THE PATHOLOGIC CHANGES IN GIANT FOLLICULAR LYMPHADENOPATHY. Arch. Path., 34:385-412. 1942.

This is usually a primary disease of the abdomen or of the abdomen and thorax combined. Whether it may arise in the spleen has not been determined. It may persist as an independent disease or may undergo transformation into polymorphous cell sarcoma. Clinically it is usually mistaken for Hodgkin's disease but the histological picture is entirely different so that diagnosis is readily made by biopsy. The disease is much more susceptible to x-rays than Hodgkin's disease, even when it has undergone sarcomatous transformation. In its earlier stages it is a comparatively innocent disorder. Even the sarcomas derived from it may likewise pursue a comparatively innocuous course for a time, but eventually they produce widespread destruction of tissues, including even invasion of bone. The disease may also undergo transformation into a variety of leukemia. There is apparently a series of disorders of the lymphoid system, including giant follicular lymphadenopathy, Hodgkin's disease, certain forms of lymphoid leukemia, lymphosarcoma, and the immature large cell sarcoma of lymph nodes, that all begin as a hyperplasia of the lymph follicle.—H. G. W.

Spleen

HERBUT, P. A., and GABRIEL, F. R. [Jefferson Med. Coll. Hosp., Philadelphia, Pa.] SECONDARY CANCER OF THE SPLEEN. Arch. Path., 33:917-921. 1942.

Among 640 persons with cancer, coming to necropsy, there were 23 instances (3.6%) of secondary involvement of the spleen. Of the splenic growths, 15 were metastatic and 8 were due either to implantation from generalized abdominal seeding or to direct extension. The explanation for the paucity of splenic metastasis is yet to be found.—H. G. W.

PINES, B., and RABINOVITCH, J. [Jewish Hosp., Brooklyn, N. Y.] HEMANGIOMA OF THE SPLEEN. Arch. Path., 33:487-503. 1942.

Only 42 cases have been reported, including the author's 6 new cases. Of these 36 were cavernous hemangioma, 1 angioma simplex, 3 hemangioma telangiectodes, and 2 hemangioblastoma. In 40% the tumors grew to large proportions but many were small and remained undiagnosed. Distribution in the sexes was equal, but the large tumors were mostly in females. Presumably, some congenital anomaly or developmental defect is responsible. In one case an adrenal tumor had metastasized to the hemangioma in the spleen.—H. G. W.

Adrenal

FORSYTHE, W. E. [New York Hosp., New York, N. Y.] BILATERAL HYPERNEPHROMA: REPORT OF A CASE. J. Urol., 47:784-786. 1942.

Another case is added to the 6 already in the literature. —H. G. W.

KEATING, F. R., Jr., and KEPLER, E. J. [Mayo Clinic, Rochester, Minn.] TUMORS OF THE ADRENAL GLAND. Surg. Clin. North Am., 21:1163-1172. 1941.

The symptoms, diagnosis, and treatment in cases of tumors of the adrenal medulla and cortex are described. —J. L. M.

NOFSINGER, C. D., and VINSON, P. P. [Lewis-Gale Hosp., Roanoke, and Med. Coll. of Virginia, Richmond, Va.] INTRABRONCHIAL METASTASIS OF HYPERNEPHROMA SIMULATING PRIMARY BRONCHIAL CARCINOMA. J. A. M. A., 119:944-945. 1942.

A case report.—H. G. W.

TAYLOR, W. N., and WISEMAN, B. CARCINOMA OF THE ADRENAL CORTEX IN A CHILD ONE YEAR OF AGE: CASE REPORT. J. Urol., 48:38-43. 1942.

An analysis is made of the 73 cases reported in the literature. In 37 instances the disease occurred in subjects over 15 years of age, and in 36 it occurred in younger persons. An additional case is reported.—H. G. W.

Pituitary

MacCALLUM, W. G. [Johns Hopkins Med. Sch., Baltimore, Md.] SUPRASELLAR TUMORS RELATED TO THE PARS INTERMEDIA OF THE HYPOPHYSIS. Arch. Path., 34:13-17. 1942.

A study of 113 tumors corresponding in structure with the pars intermedia. These tumors usually lie above the diaphragm of the sella turcica, although frequently they extend down to compress the hypophysis. It cannot be said that the tumor actually springs from the thin layer of cells that forms the ordinarily recognizable pars intermedia, but rather, perhaps, from that same type of cell, which is distributed along the stalk or even up into the pars tuberalis. Microscopically the structure is remarkably uniform, the cells tending to be rather cylindric in form around capillaries, and often there are irregular spaces between the strands of cells. Nothing in the way of specific granules can be brought out by special stains. The tumor must be differentiated from chromophobe adenoma, which usually arises in the anterior lobe of the hypophysis and is composed of cells differently arranged from those in the suprasellar tumors. The symptoms are usually disturbance of vision with bilateral hemianopsia and often complete blindness of one eye, loss of libido, beginning obesity, and other symptoms resembling Fröhlich's syndrome. In the same position adamantinomas also are occasionally found. —H. G. W.

PERKINS, C. W. [Norwalk, Conn.] CRANIOPHARYNGIOMA. TUMOR OF THE HYPOPHYSIAL DUCT. Connecticut M. J., 6:94-98. 1942.

A case report with discussion.—R. C. R.

van BUCHEM, F. S. P. [St. Elisabeth-Krankenhaus, Tilburg, Holland] DIE CUSHINGSCHE KRANKENHEIT. [CUSHING'S DISEASE.] Acta Med. Scandinav., 108:544-560. 1941.

The course of Cushing's disease was followed for 8 years prior to the patient's death. All the characteristic

manifestations, with the exception of osteoporotic changes, were present, and a basophilic pituitary adenoma was found at necropsy.—M. J. E.

Thyroid

BONNE, C. [Inst. of Path., Sch. of Med., Batavia, Java] OVER MALIGNE SCHILDKLIERGEZWELLEN. [MALIGNANT TUMORS OF THE THYROID.] South African M. J., 15:147-152. 1941.

The estimation of malignancy in a surgically removed nodular goiter, or in a biopsy of a nodule from it, is, on the whole, a thankless task for the pathologist. Even if the histological structure seemed completely benign or even normal (struma parenchymatosa or colloides) the thyroid tissue may, in the long run, show malignant characteristics in the form of metastases at a distance, especially in the bones. Such metastasizing goiters are described here under the name of malignant adenoma.

The pathologist can offer a positive contribution to the estimation of the malignancy of a nodular goiter in the case of goiters with a papilliferous growth. Experience teaches that these papilliferous tumors of the thyroid often show local signs of malignancy in the form of infiltration into adjacent organs, metastases in the neighboring cervical lymph glands, and local recurrence. Distant metastases are exceptional in these cases.

The clinical and pathological diagnosis of malignant adenoma is generally hardly possible until distant metastases are recognized. The clinical diagnosis of probable papilliferous thyroid tumor (struma papillaris) can sometimes be made on the grounds of close adhesions between the untreated tumor and adjacent organs, and can be confirmed by histological examination. In other cases microscopic examination unmasks the true nature of the tumor before it can be recognized clinically. Struma papillaris often develops in aberrant thyroid tissue lying in the side of the neck, and is then easily mistaken for tuberculosis of the cervical lymph glands, a tumor of a cervical lymph gland, lymphogranuloma, or a salivary gland tumor. Struma papillaris must be treated as a malignant tumor.—Author's abstract. (A translation supplied by the Royal Society of Medicine.—E. L. K.)

JOBE, M. C. [Denver, Colo.] PARATHYROID TUMORS CAUSING HYPERCALCEMIA. Rocky Mountain M. J., 38: 121-126. 1941.

The findings on physical examination, blood studies, and x-ray examination in cases of parathyroid tumors with hypercalcemia were presented. A case report of this type of tumor was included. The anatomy of the parathyroid and its surgical approach were considered.—R. C. R.

MEYER, K. A., and RAGINS, A. B. [Chicago, Ill.] CARCINOMA OF THE PARATHYROID. POSTMORTEM STUDIES. Proc. Inst. Med. Chicago, 13:434-435. 1941.

A case report with postmortem studies.—R. C. R.

WIKLE, H. T., and RITZMANN, A. J. [Cumberland Hosp., Brooklyn, N. Y.] THE COURSE OF CARCINOMA OF THE THYROID GLAND. REPORT OF AN UNUSUAL CASE. Am. J. Surg., 56:507-512. 1942.

A case is described of a diffuse adenocarcinoma of the thyroid in a man of 81. The tumor recurred after operation, causing death in $4\frac{1}{2}$ months.—H. G. W.

Miscellaneous

BECKER, S. W., KAHN, D., and ROTHMAN, S. [Univ. of Chicago Sch. of Med., Chicago, Ill.] CUTANEOUS MANIFESTATIONS OF INTERNAL MALIGNANT TUMORS. Arch. Dermat. & Syph., 45:1069-1080. 1942.

The cutaneous manifestations in two patients with internal neoplasms are described; a survey of cutaneous manifestations accompanying malignant tumors of internal origin is presented and the possible relation of the lesions is discussed.—H. G. W.

CALLISTER, A. C. [Salt Lake City, Utah] HYGROMA COLLI CYSTICUM. Rocky Mountain M. J., 38:562-564. 1941.

A short discussion of the literature, pathology, and treatment of the disease, with the report of a case.—R. C. R.

MACKLIN, M. T. [London, Canada] HAS A REAL INCREASE IN LUNG CANCER BEEN PROVED? Ann. Int. Med., 17:308-324. 1942.

The data that are used to support the claim that lung cancer has increased faster than other forms of cancer are inadmissible for that purpose, since it is not known what proportion of lung cancer cases were unrecognized formerly or are so today. It can be stated only that diagnosed lung cancer is increasing at a rate which appears to be faster than that of other diagnosed cancers. Suggestions are made as to precautions to be taken in analyzing statistics on lung cancer. It is recommended that the search for environmental factors supposed to be the basis of the unduly great increase in lung cancer should await further proof that the increase in incidence has been as spectacular as has been stated.—H. G. W.

SWEETSER, T. H. [Minneapolis General Hosp., Minneapolis, Minn.] RETROPERITONEAL TUMORS INFLUENCING THE KIDNEYS AND URETERS. J. Urol., 47:619-631. 1942.

A clinical report of 7 cases.—H. G. W.

SYMPOSIUM ON LARGE BOWEL CANCER, FROM 16TH CLINICAL CONGRESS, CONNECTICUT STATE MEDICAL SOCIETY. Connecticut M. J., 5:890-896. 1941.

Papers were presented by J. F. Burke, W. Mendelsohn, and A. W. Oughterson.

An analysis of a series of cases in seven hospitals of three Connecticut cities indicates that delay in operation is the factor most responsible for a poor prognosis in carcinoma of the large bowel and rectum. The data presented on operative mortality, operability rate, and operation rate compare well in general with those supplied by various other clinics. It is suggested that more patients whose tumors are still operable be submitted to radical procedures, and that both physicians and surgeons attempt to reduce the delay in diagnosis and operation.—R. C. R.

TUTA, J. A., and APFELBACH, G. L. [Grant Hosp., Chicago, Ill.] PAPILLARY ADENOCYSTOMA LYMPHOMATOSUM OF THE NECK. Am. J. Surg., 56:504-506. 1942.

A case report.—H. G. W.

WALLIS, O. [Chicago, Ill.] THE ROLE OF THE FALLOPIAN TUBES IN THE SPREAD OF PELVIC CARCINOMA. Proc. Inst. Med. Chicago, 13:295-296. 1941.

A case report with discussion of the invasion and dissemination of carcinomatous cells into and through the fallopian tubes to the abdominal cavity.—R. C. R.

Book Reviews

CYTOLOGY AND CELL PHYSIOLOGY. Edited by Geoffrey Bourne. Clarendon Press, Oxford. XII + 296 pages.

In the Preface the Editor states that this volume represents an attempt to bring together chemical, physicochemical, and morphological aspects of the study of cells, and this has been carried out by selecting a number of subjects which are representative of different fields of the study of cells and which relate, as far as possible, one to the other. The subjects of the various chapters together with their authors are as follows: Technique for the Study of Cell Structures, by J. R. Baker; General Methods for the Physical and Physicochemical Study of Cells, by J. F. Danielli; The Monolayer Technique, by J. H. Schulman; Cell Membranes, by J. F. Danielli; Mitochondria and Golgi Apparatus, by G. Bourne; Nucleus, Chromosomes, and Genes, by M. J. D. White; Micro-Incineration, by E. S. Horning; Enzyme Systems, by H. Blaschko and W. Jacobson; and Pathological Cytology, by R. J. Ludford.

Since the central problem of cancer research is the investigation of the aberration of cellular functioning which determines malignancy, the topics discussed may be of general interest to cancer workers. Malignant cells are specifically discused in two of the chapters. E. S. Horning describes the present state of our knowledge concerning their inorganic constituents and R. J. Ludford contributes a brief review of their general cytological characteristics.

R. J. Ludford

BLOOD GROUPING TECHNIC. By F. Schiff and W. C. Boyd. Interscience Publishers, Inc., New York. 1942. 248 pages. Price $5.00.

This useful manual by the late Dr. Schiff and by Dr. Boyd gives in concise and readily understandable form the useful aspects of blood grouping. There is a succinct but satisfactory account of the various blood grouping methods, with a discussion of the M and N types. The material on the Rh factor is quite adequate. Of use to those interested is the section on blood transfusion, including as it does a description of the means of investigating transfusion accidents. The New York City regulations regarding blood donors and blood banks are given in full, as amended in 1939. A useful item for a second edition of the book would be the O.C.D. blood bank procedures.

An adequate section on the forensic aspects of blood grouping, with a discussion of disputed paternity, is given. There is also a description of the forensic application of blood grouping, including the study of blood stains.

In addition, a useful chapter is that on antigens similar to those of blood groups found in animals. A final section on the anthropological applications of blood grouping is clearly presented.

The reviewer feels that this book can be highly recommended to those who already have some fundamental knowledge of the problems involved in blood grouping and blood bank management.

Shields Warren

LE PROBLEME DU CANCER. By Charles Oberling. Collection "France Forever." L'Arbre, Montreal. 1942. 300 pages. Price $1.50.

The geneticist moves among his animals as though endowed with a spark of divine power, for he can determine the course of their lives even before they are born. He shows us strains bred for longevity, since by judicious matings over many generations he has eliminated all those weaknesses upon which disease could lay hold, and so can guarantee a life span of more than ordinary length. Then he shows us other strains that are resistant to cancer, and finally still others in which the disease will appear in all the members, at a certain fixed time and as inevitably as puberty or the menopause.

This abridged translation of one of the opening paragraphs in the section on heredity furnishes an example of the easy and entertaining style in which Oberling's book is written.

Avowedly for the layman, it can but sketch the general course of cancer research, and the specialist will therefore pass rapidly through the pages on theory, on transplanted tumors, and on the experimental induction of neoplasms, for their contents will be thoroughly familiar to him.

He may be tempted to pause, however, over some unusually felicitous passage such as the discussion of the role played by irritation in etiology. Infatuation with it has led to what might be called the hypnosis of positive cases, and to the total neglect of innumerable negative ones. Cancer is so relatively rare that it must be the outcome of an extraordinary coincidence of favorable circumstances, thus resembling somewhat the winning number in a lottery, and the irritation hypothesis accordingly becomes the hypothesis of chance.

That such diverse compounds as the carcinogenic hydrocarbons and aromatic amines, hormones, hydrochloric acid, sugar, and salts of zinc should elicit malignant growth may be explained by the supposition that they modify the tissues in such a way as to permit the intervention of an unrecognized carcinogen already present there. Certainly they do not achieve their effect by irritation alone.

From here the argument passes logically to a discussion on virus etiology, much the most interesting part of the book to students of cancer, for whether or not they are able to accept such an explanation they will admit that the evidence for and against this vexed question is presented fully, fairly, and entertainingly.

A long road has been traversed, says Oberling, since Borrel first suggested the virus hypothesis. Though the evidence available at that time was flimsy in the extreme, subsequent investigations have disclosed leukemias and benign and malignant growths due to virus infection, and the discovery of a papilloma of the rabbit and an adenocarcinoma of the frog kidney, both caused by viruses, has finally broken down the barrier arbitrarily erected between the tumors of birds and those of other species. Furthermore, and this is highly significant, it has been shown that a neoplasm may be caused by a virus and yet resist all attempts at its recovery. Thus it is no longer justifiable to deny the presence of virus in a tumor that cannot be transmitted by cell-free filtrates made according to current methods.

One characteristic of the viruses in particular is of the

greatest interest. They act almost instantly, whereas the other carcinogens so far recognized exert their effect only after a long preparatory period. These latter, then, seem to be indirect, whereas the viruses are direct, causes.

When we reflect on the diversity of all these agents we have the sensation of knowing at once too much and too little. Too much because instead of a single solution of the problem a whole series of explanations is offered; too little because of our profound ignorance of the cell, where all causes terminate. We occupy the position of one who is asked to explain the working of an automobile from its external appearance alone. He may analyze what is put into the machine to make it go, and what comes out of it, but he may not raise the hood. He may ignite or dissolve the vehicle to determine its total content of metals and combustible material, but that is all. How can he possibly form any idea of the motor, or of the thousand and one accessories essential to function? Yet the cell is infinitely more complex, and the derangement that we call cancer is hidden away deep in some inaccessible part of the mechanism.

Though the secondary, or extrinsic, causes are so many, they must be analyzed with the greatest care, for even though they be indirect complete familiarity with them would lead to prevention. Thus the radiologist says that roentgen cancer is caused by x-rays, and he knows that by taking suitable precautions he can prevent it; for him the question is settled.

But for the investigator in the laboratory the question is not settled. For him the x-ray is but an adjuvant to some other and still wholly unrecognized influence. For him, cancer is always the same disease, no matter what its secondary cause, for whether the malignant cell be found in fowl, rabbit, or man it has always the same features—exalted proliferation, invasive growth, and, above all, autonomy.

But what is the intrinsic cause? It must be: (a) carcinogenic; (b) present in every tumor; (c) compatible in its activities with, and explanatory of, all the secondary causes.

Radiant energy, parasites, and heredity may be eliminated at once, for they are not the cause of every cancer. There remain the carcinogenic chemicals and the viruses. Invariable presence is theoretically possible for either group, but if the carcinogenic compounds were the true cause they would be involved in the genesis of virus tumors and it would have to be admitted that the viruses in themselves are incapable of initiating cancer, acting only indirectly by forming carcinogenic chemical compounds. This contradicts facts already established, for the viruses elicit their effects more rapidly than the compounds they would elaborate.

Priority rests, therefore, with the viruses, but in assigning them this role we enter the domain of pure speculation. In examining the evidence for and against them we must see whether they fulfill the three postulates set forth above.

Discussion of the first is unnecessary, for many viruses are known that produce benign or malignant tumors.

The second postulate demands the constant presence of a virus in every neoplasm. Now if viruses are responsible for all tumors they would necessarily be concerned in the inception of those induced by chemical compounds, and the assumption would have to be made that every animal susceptible to these agents harbored the viruses of all tumors that might arise under their influence. This is not so unreasonable as it appears to be at first sight, for does not everyone carry in his digestive tract the colon bacillus and innumerable other organisms? What holds for bacteria ought to be true of viruses, the more so because these latter, obligate intracellular parasites as they are, lie sheltered from all the reactions that the body sets up against the bacteria. Nay more, do not the viruses furnish the best example of extraordinarily wide distribution, affecting all of certain varieties of plants and certain species of animals?

But here another difficulty appears. As the types of neoplasia are legion, and each virus elicits in general the same kind of tumor, there would have to be granted not only a wide distribution but a surprising multiplicity of viruses. Well, Nature has never been niggardly in such matters, and nothing is more amazing than the prodigious number of varieties included in certain species. Nearly 7,000 varieties of microbe take part in putrefaction, so why not allow a much smaller number of cancer viruses? In any case the total may not be so extravagant after all, for viruses may alter their cytotropism on occasion, those of fowl leukemia and fowl sarcoma, for example, giving rise to a wide variety of neoplastic changes.

If viruses are responsible for all tumors, why is it that they cannot be recovered invariably? Perhaps the effort fails because the virus has entered so completely into the economy of the cell as to become in effect an inseparable part of it; perhaps because the virus is so exacting in its demands that animals into which a filtrate is injected do not offer a favorable soil and thus the virus remains undemonstrable, even though present. Neoplasms that can be easily transmitted by cell-free extracts would then represent infections that are not too strictly conditioned, and it is highly probable that they are exceptions.

We come now to the third and last postulate, according to which the virus hypothesis must conform with all that is known respecting carcinogenesis, and here we shall examine the relation of viruses to cancers associated with parasitic infestation, carcinogenic compounds, and hereditary influences.

The effect of parasites is so inconstant that they alone cannot be a cause of cancer, and Borrel was therefore led to assume the intervention of a virus, relegating the parasite itself to the subordinate role of inoculator. The propagation of a virus disease by a parasite is no mere figment of the imagination, for recent investigations of swine influenza show that it does occur; hence the virus hypothesis is not inconsistent with the production of cancer by parasites.

The idea that a virus may be responsible for tumors that follow the injection of carcinogenic chemicals seems at first sight less plausible, and has been called even absurd. Yet recent investigations have brought out some singularly suggestive findings. For example, the virus of a rabbit papilloma, introduced into the blood stream, attacks the superficial epithelium only if this has been painted with tar or benzpyrene. In such a case it induces malignant transformation much more promptly than either one alone, and the carcinomas are more malignant than those following the application of tar or benzpyrene, for

these carcinogenic agents have sensitized the epithelium to the virus. Thanks to this and to other observations the intervention of viruses in the production of tumors by chemical compounds no longer seems improbable, and the virus hypothesis is not incompatible with the known facts.

As for inherited influences, cancer arises with extraordinary regularity in certain strains of mice, where it appears so inevitably determined by heredity that the mediation of a virus seems hardly conceivable. Actually, it cannot be excluded. No one will deny that susceptibility to some infections may be associated with hereditary characteristics, and if the virus of St. Louis encephalitis attacks certain mouse strains with perfect selectivity why deny the same property to a carcinogenic virus?

Furthermore, when it became known that maternal influence is much more effective than paternal in determining susceptibility to mammary carcinoma in mice, a mendelian explanation was no longer tenable. Borrel had already suspected that the assumed heredity might be connected with the passage of a carcinogenic virus from mother to offspring through the milk, and accordingly he exchanged newborn mice from mothers of cancer and noncancer strains. The outcome seemed to confirm his suspicion, though the data are difficult to evaluate because his strains were not truly homozygous. It has been fully substantiated, however, by more recent investigations upon unexceptionable material, and there is no longer any doubt that some factor of the highest significance, which affects the entire organism, is transmitted with the milk. Many believe it to be a virus, but the evidence is not yet complete. In any case the virus hypothesis does not contravene what is known of heredity.

A few matters still remain for discussion. The virus hypothesis is often declared unacceptable because cancer is not contagious. But neither are the leukemias and the filterable sarcomas of fowls, though each bird affected carries virus enough to infect millions of others.

Most of the objections, indeed, depend upon the outmoded idea that an infectious agent attacks indiscriminately and always, immediately eliciting its disease. Thus is it said that constant presence and wide distribution of cancer viruses would be inconsonant with the appearance of cancer at certain periods of life. It should be remembered, however, that some infections actually are conditioned by age. Ringworm of the scalp, for instance, is seen as a rule only in children and disappears promptly at puberty, but testicular atrophy creates anew a favorable soil. No chemist has ever discovered the changes taking place in the hair follicles under the influence of puberty; the fungus of ringworm is more knowing.

The only serious reproach to the virus hypothesis is that it is still unproved.

Most students of cancer therefore prefer an explanation less open to criticism, the hypothesis of somatic mutation. Its great virtue is that a way is left open for any etiological conception, since mutations may arise from causes the most diverse; its great fault, that it explains nothing. Somatic mutations, observed principally in plants and insects, are exceptionally rare in mammals, and those actually known affect secondary characteristics like pigmentation; they never bring about such an upheaval as the malignant transformation of a cell. Nor do cancers in general arise suddenly, as do mutations, but only after a long preparatory period of cellular derangement.

The cytological disorders that usually precede the appearance of cancer are hard to reconcile with the idea of simple somatic mutation. Many precancerous lesions include cells that appear to be already malignant, yet the true neoplastic change may be delayed for years. At what point in this process does the mutation occur? If at the end, of what sort were the preliminary changes? If at the beginning, not one mutation, but a whole series, would have to be assumed.

The most serious objection to the mutation hypothesis, in fact, is the existence of the virus tumors. For as viruses are able to initiate neoplastic growth much more rapidly than any other agent mutation need not be invoked to explain them.

To those who may be disposed to chide the author for a too enthusiastic partisanship he replies that it is never ill judged to be guided by a hypothesis so long as it does not do violence to the known facts, and that the best proof of its value is the amount of research to which it gives rise. Submitted to these tests the virus hypothesis has nothing to fear.

For him, as the Preface says, it is neither a preconceived idea nor yet a part of his medical background. Masson, his first teacher and a cytologist of note, always preferred a cellular theory, whereas Roussy had a horror of partisanship, and so ardent was his desire to retain the kernel of truth in every hypothesis that he was inevitably led to accept a plurality of causes and to seek the final cause in mutation. Borrel, on the other hand, was of a totally different temperament. He defended his ideas passionately in spite of everything, sometimes even against the evidence, and such seemed to his associates to be the case with cancer. Oberling, and many others, listened to his arguments, admiring his seductive eloquence and his fertile imagination, but without believing one single word of his tales about viruses that were ubiquitous and able to work their way along from one cell to the next until, at some auspicious moment, they encountered a susceptible one and ended by initiating cancer.

If the words of Borrel awoke no echoes it was because they were uttered at a time when it needed the greatest courage to maintain the virus hypothesis. Yet he was able to forsee that some experiments with spiroptera would have a negative outcome, that parasites might carry viruses, and that in the so called hereditary cancer of the mouse something other than hereditary influences must be transmitted. And more than once he was heard to say that it must pass by way of the milk.

Well, all these predictions have been realized, says Oberling, and one cannot help being impressed thereby.

But although the virus hypothesis does offer a plausible explanation for neoplastic growth, it is as a logical postulate rather than as an actual demonstration. The hypothesis is not in flagrant disagreement with the facts, though perhaps this can no longer be said tomorrow. Yet even though our conceptions should have to be revised almost overnight there would be the consolation, after all, that we are not seeking to confirm an idea but to solve a problem. In this quest all are likely to go astray, for, as Fontana wrote, in 1781, research is a game of chance in which the probability of error is great and that of discovering the truth small. WILLIAM H. WOGLOM